(51) International Patent Classification 6 : C07D 257/02, 493/10, 405/14, A61K 49/00, C07F 9/6524 // (C07D 493/10, 317:00, 317:00)	A1	(11) International Publication Number: **WO 95/31444** (43) International Publication Date: 23 November 1995 (23.11.95)

(54) Title: ENHANCED RELAXIVITY MONOMERIC AND MULTIMERIC COMPOUNDS

(57) Abstract

Metal chelates capable of exhibiting an immobilized relaxivity between about 60 and 200 mM^{-1}s^{-1}/metal atom are useful as magnetic resonance imaging agents. Additionally, a compound which is useful as a metal-chelating ligand has formula (II): wherein Q is a 4- to an 8-membered carbocyclic ring which may be fully or partially saturated; t is an integer from 2 to 16; each R group is independently hydrogen, -OH, -CH$_2$-A, -OCH$_2$CH(OH)CH$_2$-A or a functional group capable of forming a conjugate with a biomolecule, provided that at least two of the R groups are selected from -CH$_2$-A or -OCH$_2$CH(OH)CH$_2$-A; and A is a moiety capable of chelating a metal atom.

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ENHANCED RELAXIVITY MONOMERIC AND MULTIMERIC COMPOUNDS

Brief Description of the Invention

In accordance with the present invention, novel monomeric and multimeric compounds having enhanced relaxivities are provided. These compounds are useful, for example, as metal-chelating ligands. The compounds are also useful in the form of metal complexes as diagnostic contrast agents. When the metal in the complex is paramagnetic, the diagnostic contrast agents are especially suitable for magnetic resonance imaging (MRI).

In one embodiment of the invention, certain specific compounds comprise a tetraazacyclododecane macrocycle, and are represented by the formula I:

I
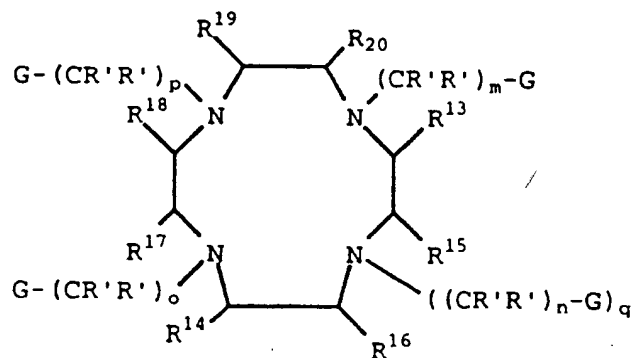
wherein

each m, n, o and p is independently 1 or 2;

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q is 0 or 1;

each G is independently -COOR'', -P(O)(OR'')$_2$, -P(O)(OR'')(R'') or -C(O)N(R'')$_2$;

each R' is independently hydrogen or alkyl,
5   alkoxy, cycloalkyl, hydroxyalkyl or aryl, each of which is optionally substituted, or a functional group capable of forming a conjugate with a biomolecule or of forming a multimer of said compound of formula I;

10   each R'' is hydrogen;

each R^{13} through R^{20} is independently hydrogen, alkyl, hydroxyalkyl, alkoxyalkyl or a functional group capable of forming a conjugate with a biomolecule or of forming a multimer of said
15   compound of the formula I;

or R^{13} together with R^{15}, and R^{17} together with R^{18}, independently form, together with the carbon atoms in the tetraazacyclododecane macrocycle to which they are attached, a fused fully or partially
20   saturated non-aromatic cyclohexyl ring which may be unsubstituted or substituted by one or more halogen, alkyl, ether, hydroxy or hydroxyalkyl groups, and which may be further fused to a carbocyclic ring, or R^{13} and R^{15} are each hydrogen and R^{17}, together with
25   R^{18}, forms a fused fully or partially saturated non-aromatic ⁄cyclohexyl ring as defined above, or R^{13}, together with R^{15}, forms a fused fully or partially saturated non-aromatic cyclohexyl ring as defined above and R^{17} and R^{18} are hydrogen; provided that
30   (a.) when G is always -COOR'' and (i.) R', R'', R^{14} and R^{16} through R^{20} are all hydrogen, then R^{13} and R^{15} are other than hydrogen; (ii.) R'' and R^{13} through R^{20} are all hydrogen, and m, n, o, p and q are each 1, then (CR'R') is other than (CH$_2$) and (CHCH$_3$); (iii.)

R', R'', R^{13}, R^{14}, R^{17} and R^{20} are all hydrogen, then at least two of R^{15}, R^{16}, R^{18} and R^{19} are other than methyl; and (iv.) R'', R^{16}, R^{19} and R^{20} are all hydrogen, and each $(CR'R')$ is independently (CHR') or (CH_2CHR'), then R^{13} and R^{15}, and R^{17} and R^{18}, are other than a fused ring; and (b.) when G is always $-P(O)(OR'')_2$, $-P(O)(OR'')(R'')$ or $-C(O)N(R'')_2$, then at least one R' or R^{13} through R^{20} is other than hydrogen;

or a salt or multimeric form thereof.

Detailed Description of the Invention

Listed below are definitions of various terms used in the description of this invention. These definitions apply to the terms as they are used throughout this specification, unless otherwise limited in specific instances, either individually or as part of a larger group.

The expression "relaxivity" refers to the effectiveness of a metal chelate to reduce the relaxation time of bulk water in contact with the metal chelate.

The expression "immobilized relaxivity" refers to the relaxivity measured when a chelate moiety can undergo only slow molecular reorientation because of rigid attachment to a large moiety or a physiological surface, or because it is dissolved in a medium of high viscosity.

The expression "water relaxivity" refers to relaxivity in water where a chelate moiety possesses a relaxivity dominated by overall molecular reorientation.

The expression "enhanced relaxivity" refers to relaxivity values made greater than those of well

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characterized prior art molecules by 1.) altering
the electronic relaxation rate, τ_s, through
modifications of the metal-donor atom bond vibration
frequencies and/or amplitudes, (this being

5   accomplished, for example, by increasing the steric
bulk and/or orientation of organic elements bonded to
the donor atoms and/or the macrocyclic carbon atoms),
2.) in a multimer by decreasing the internal
molecular motion of one monomer unit relative to

10   another (this being accomplished, for example, by
increasing the steric bulk of the organic groups
linking the monomer units) or 3.) by decreasing the
molecular reorientation of a monomer or a multimer
attached to a large moiety or a physiological

15   surface.

The term "stability" refers to the equilibrium
formation constant (K) of the reaction $M+L \rightarrow M(L)$
where $K=[M(L)]/[M][L]$, M is a metal ion, L is a
chelating ligand and M(L) is a chelate complex of a

20   metal and a ligand.

The term "alkyl" refers to both straight and
branched, unsubstituted chains of carbon atoms.
Those chains having 1 to 5 carbon atoms are
preferred. Methyl is the most preferred alkyl group.

25   The term "cycloalkyl" refers to cyclic
hydrocarbon groups of 3 to 8 carbon atoms. The
groups may be unsubstituted or substituted by, for
example, alkyl, halogen, hydroxy, hydroxyalkyl,
alkoxy, alkanoyl, alkanoyloxy, amino, alkylamino,

30   dialkylamino, alkanoylamino, thiol, alkylthiol,
nitro, cyano, carboxy, carbamoyl, alkoxycarbonyl,
alkylsulfonyl, sulfonamido and the like.

The term "alkoxy" refers to -alkyl(O).
Methoxy is the most preferred alkoxy group.

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The term "aryl" refers to phenyl, pyridyl, furanyl, thiophenyl, pyrrolyl, imidazolyl and the like, all of which may be substituted. Preferred substituted aryl groups are those substituted with 1,
5 2 or 3 halogen, nitroamino, maleimido, isothiocyanato, hydroxy, hydroxyalkyl, alkyl, alkoxy, carbamoyl, carboxamide, acylamino or carboxy moieties.

"Hydroxyalkyl" refers to straight and branched
10 alkyl groups including one or more hydroxy radicals such as $-CH_2CH_2OH$, $-CH_2CH_2OHCH_2OH$, $-CH(CH_2OH)_2$ and the like. (See, for example, Sovak, M., Editor, Radiocontrast Agents, Springer-Verlag, 1984, pp. 1-125).

15   The term "aralkyl" refers to an aryl group bonded through an alkyl group.

The term "carbocyclic ring" refers to a ring system in which all the ring atoms are carbon, e.g., phenyl or cyclohexyl. The ring may be unsubstituted
20 or substituted by, for example, alkyl, halogen, hydroxy, hydroxyalkyl, alkoxy, alkanoyl, alkanoyloxy, amino, alkylamino, dialkylamino, alkanoylamino, thiol, alkylthiol, nitro, cyano, carboxy, carbamoyl, alkoxycarbonyl, alkylsulfonyl, sulfonamido and the
25 like.

The term "halogen" refers to bromo, chloro, fluoro or iodo.

The term "alkanoyl" refers to the group alkyl-C(O)-.

30   The term "alkanoyloxy" refers to the group alkyl-C(O)-O-.

The term "amino" refers to the group $-NH_2$.

The term "alkylamino" refers to the group $-NHR$ where R is alkyl.

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The term "dialkylamino" refers to the group -NRR' where R and R' are each, independently, alkyl.

The term "alkanoylamino" refers to the group alkyl-C(O)-NH-.

The term "thiol" refers to the group -SH.

The term "alkylthiol" refers to the group -SR where R is alkyl.

The term "nitro" refers to the group -NO$_2$.

The term "cyano" refers to the group -CN.

The term "carboxy" refers to the group -C(O)OH or the group -C(O)OR where R is alkyl.

The term "alkoxycarbonyl" refers to the group alkoxy-C-(O)-.

The term "alkylsulfonyl" refers to the group alkyl-SO$_2$-.

The term "sulfonamido" refers to the group -SO$_2$NH$_2$, the group -SO$_2$NHR or the group -SO$_2$NRR' where R and R' are each, independently, alkyl, cycloalkyl or aryl.

The term "carbamoyl" refers to the group -C(O)NH$_2$, the group -C(O)NHR or the group -C(O)NRR' where R and R' are each, independently, alkyl, alkoxy or hydroxyalkyl.

The term "carboxamide" refers to the group -C(O)NH$_2$, the group -C(O)NHR or the group -C(O)NRR' where R and R' are each, independently, alkyl.

The term "acylamino" refers to the group -NH-C(O)-R where R is alkyl.

The expressions "bioactive group" and "bioactive moiety" denote a group which is capable of functioning as a metabolic substrate, catalyst or inhibitor, or is capable of being preferentially taken up at a selected site of a subject, such as by

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possessing an affinity for a cellular recognition
site.

　　　When compounds of the formula I are in the
multimeric form, each monomer is preferably linked by
5   a cyclic bridging group represented by the general
formula II:

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II
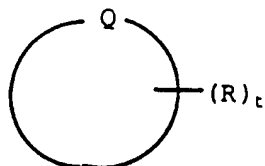
wherein

Q is a 4- to an 8-membered carbocyclic ring
which may be fully or partially saturated;

t is an integer from 2 to 16;

each R group is independently hydrogen, -OH,
-CH$_2$-A, -OCH$_2$CH(OH)CH$_2$-A or a functional group
capable of forming a conjugate with a biomolecule,
provided that at least two of the R groups are
selected from -CH$_2$-A or -OCH$_2$CH(OH)CH$_2$-A; and

A is the monomer of formula I.

Of course, the cyclic bridging group may be used to
link known moieties as well. Where it is a known
moiety which is being linked by the cyclic bridging
group of formula II, A is any moiety capable of
chelating a metal atom.

Preferred bridging groups of the formula II
are those wherein
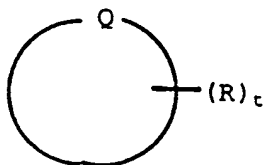
is a compound of the following formula III:

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R^1 R^2 R^3 R^4

R^{12}

R^5

R^6 .

R^{11}

R^{10} R^9 R^8 R^7
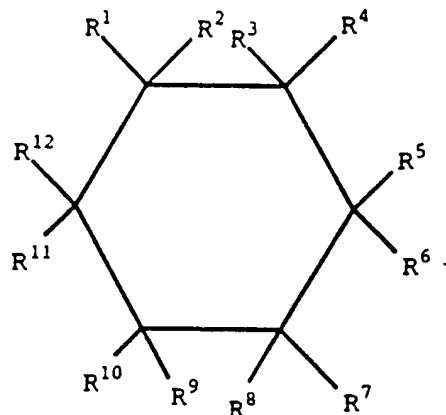
wherein

each R^1 through R^{12} group is independently hydrogen, -OH, -CH$_2$-A, -OCH$_2$CH(OH)CH$_2$-A or a

5   functional group capable of forming a conjugate with a biomolecule;

at least two of R^1 through R^{12} are selected from -CH$_2$-A or -OCH$_2$CH(OH)CH$_2$-A;

R^8 and R^9 taken together may additionally form

10   the group -O-[C(RR)]-O- where each R is independently hydrogen or alkyl, or R^8 and R^9 taken together may form

(Chemical structure with substituents R^{10}, R^7, R^{11}, R^6, R^{12}, R^5, R^1, R^2, R^3, R^4)

;
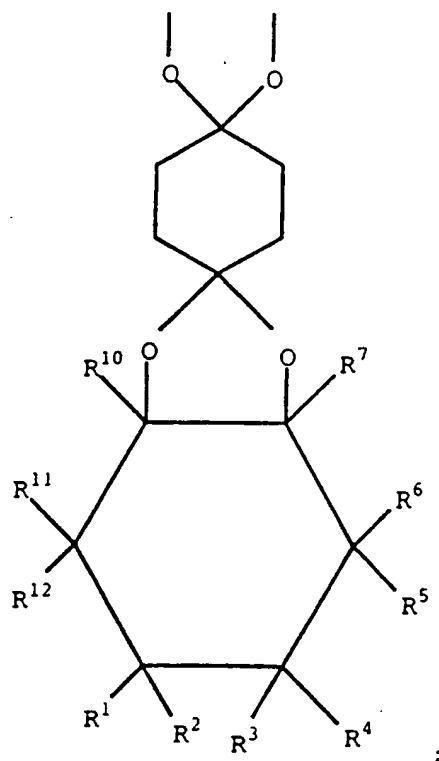
and

A is a moiety described above.

When A is a known monomer, for example, A is preferably
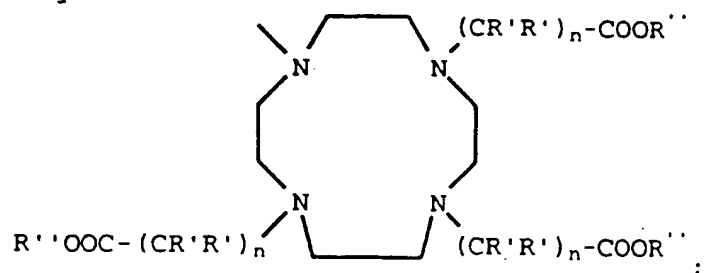
(Chemical structure: macrocyclic tetraaza ring with substituents $(CR'R')_n$-COOR'', R''OOC-$(CR'R')_n$, $(CR'R')_n$-COOR'')

;

wherein

each R' is independently hydrogen, alkyl, alkoxy, hydroxyalkyl, aryl, aralkyl or arylalkoxy;

each R'' is hydrogen; and

each n is 1 or 2;

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or a salt thereof.

Contrast agents with significantly enhanced relaxivities are of great interest, not only because they offer improved efficacy at reduced doses for
5   current clinical applications, but also because they may provide the sensitivities needed for imaging various biochemical processes.

Certain preferred compounds having enhanced relaxivities are (i.) chelates possessing a stability
10   greater than or equal to 10^{15} M^{-1} and capable of exhibiting an immobilized relaxivity between about 60 and 200 $mM^{-1}s^{-1}$/metal atom, for example between about 70 and 150 $mM^{-1}s^{-1}$/metal atom, or between about 80 and 100 $mM^{-1}s^{-1}$/metal atom; and (ii.) multimeric chelates
15   possessing monomer units with a stability greater than or equal to 10^{15} M^{-1}, and having relaxivity values (not immobilized) greater than 5$mM^{-1}s^{-1}$/metal atom.

In designing new chelates with these elevated
20   relaxivities, the inventors have noted that immobilized relaxivity can depend strongly, for example, on the structure of the chelate. Without being bound by any particular theory, apparently a mechanism that involves rigidifying the chelate
25   structures in solution with, for example, alkyl substitutions on the tetraaza ring of chelates, especially when the substitution is introduced into the carboxylate arms of the chelates, affects immobilized relaxivity. In the Solomon-Bloembergen-
30   Morgan (SBM) model, the electronic relaxation of a paramagnetic metal complex is viewed as occuring through a dynamic modulation process about the transient zero-field splitting (ZFS) of the metal's electronic spin levels. This transient ZFS is

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induced by the structural distortion of the metal
complex from its ideal symmetry in solution which is
thought to be caused by its collision with solvent
molecules.   Alkyl substitution on either the tetraaza
5   ring or the carboxylate arms of a chelate is thought
to reduce the flexibility of the chelate for
structural distortion in solution. This in turn
reduces the magnitude of ZFS, giving an increased
immobilized relaxivity value.
10   Water relaxivity is generally determined in
aqueous Bis Tris buffer (pH 7) solutions by the
standard inversion-recovery method (known to those in
the art) at 20 MHz and 40°C. (See, e.g., X. Xhang,
Inorganic Chemistry, 31, 1992, 5597, the entire
15   contents of which are hereby incorporated by
reference.) While a mathematical description of the
relaxation mechanism at the presence of a
paramagnetic species is provided by the classical SBM
equations, it is experimentally difficult to explore
20   the dependence of relaxixity on structure because the
relaxivity values of most low-molecular weight
chelates are often controlled by their rapid tumbling
motions in regular aqueous solutions.
           To eliminate the overriding effect of
25   molecular tumbling in regular aqueous solutions, the
relaxivity of a chelate can be determined in aqueous
sucrose solutions or other media that result in a
reduction of molecular reorientation of any solute.
In these solutions, the relaxivity values
30   approximate, under optimal conditions, the
relaxivities of chelates in biologically immobilized
systems such as those covalently attached to cell
surfaces. Hence, these values in sucrose solutions
are defined as immobilized relaxivity. Immobilized

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relaxivity is generally determined under the same
conditions as water relaxivity (see supra) except
that the viscosity of the solutions is increased to
80 cp by the addition of solid sucrose to the aqueous
5   solution of chelate, and the temperature is set at
20°C. In the calculation of relaxivity, one uses the
concentration of the solute in the aqueous solution
(before sucrose is added).

         When used for relative comparison, this method
10   serves as a simple screening technique for
differentiating chelates designed for efficient
biological targeting.

         Thus, the compounds of the invention, including
compounds of the formula I and formula II, and salts
15   thereof, may be complexed with a paramagnetic metal
atom and used as relaxation enhancement agents for
magnetic resonance imaging. These agents, when
administered to a mammalian host (e.g., a human)
distribute in various concentrations to different
20   tissues, and catalyze relaxation of protons (in the
tissues) that have been excited by the absorption of
radiofrequency energy from a magnetic resonance imager.
This acceleration of the rate of relaxation of the
excited protons provides for an image of different
25   contrast when the host is scanned with a magnetic
resonance imager. The magnetic resonance imager is
used to record images at various times, generally
either before and after administration of the agents,
or after administration only, and the differences in
30   the images created by the agents' presence in tissues
are used in diagnosis. In proton magnetic resonance
imaging, paramagnetic metal atoms such as
gadolinium(III), and manganese(II), chromium(III) and
iron(III) (all are paramagnetic metal atoms with

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favorable electronic properties) are preferred as
metals complexed by the ligands of the invention,
including the ligands of formula I and formula II.
Gadolinium(III) is the most preferred complexed metal
5   due to the fact that it has the highest paramagnetism,
it has low toxicity when complexed to a suitable
ligand, and it has high lability of coordinated water.
When the distance between the monomeric gadolinium
chelate units in a complex is at least about 6
10   angstroms, the complexes tend to be sufficiently
stable. Those compounds of formula II, when complexed
with gadolinium (III) ions, are particularly useful.
The distance between the monomeric gadolinium chelate
units in these complexes is generally greater than 6
15   angstroms (although in certain circumstances a distance
of 4.5 angstroms is sufficient), and the rigid bridges
of these complexes assist in reducing independent
motion of the gadolinium ions.
        The metal-chelating ligands of the present
20   invention can also be complexed with a lanthanide
(atomic number 58 to 71) and used as chemical shift
or magnetic susceptibility agents in magnetic
resonance imaging or in magnetic resonance _in vivo_
spectroscopy.
25   While the above-described uses for the metal-
chelating ligands of the present invention are
preferred, those working in the diagnostic arts will
appreciate that the ligands can also be complexed
with the appropriate metals and used as contrast
30   agents in other imaging techniques such as x-ray
imaging, radionuclide imaging and ultrasound imaging,
and in radiotherapy.

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<u>Use in Imaging</u>

To use the ligands of the present invention for imaging, they are first complexed with an appropriate metal. This may be accomplished by

5   methodology known in the art. For example, the metal can be added to water in the form of an oxide or in the form of a halide or acetate and treated with an equimolar amount of a ligand of the present invention. The ligand can be added as an aqueous

10   solution or suspension. Dilute acid or base can be added (where appropriate) to maintain a suitable pH. Heating at temperatures as high as 100°C for periods of up to 24 hours or more may sometimes be employed to facilitate complexation, depending on the metal

15   and the chelator, and their concentrations.

Pharmaceutically acceptable salts of the metal complexes of the ligands of this invention are also useful as imaging agents. They can be prepared by using a base (e.g., an alkali metal hydroxide,

20   meglumine, arginine or lysine) to neutralize the above-prepared metal complexes while they are still in solution. Some of the metal complexes are formally uncharged and do not need cations as counterions. Such neutral complexes may be preferred

25   in some situations as intravenously administered x-ray and NMR imaging agents over charged complexes because they may provide solutions of greater physiologic tolerance due to their lower osmolality.

The present invention also provides

30   pharmaceutical compositions comprising a compound of the invention, including a compound of the formula I or II, or a salt of one of these compounds, optionally complexed with a metal, and a pharmaceutically acceptable vehicle or diluent. The

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present invention further provides a method for
diagnostic imaging comprising the steps of
administering to a host a compound of the invention,
or a salt thereof, which is complexed with a metal,
5   and obtaining a diagnostic image, preferably a
magnetic resonance image, of said host.

      Sterile aqueous solutions of the chelate
complexes of the present invention are preferably
administered to mammals (e.g., humans) orally,
10   intrathecally and, especially, intravenously in
concentrations of about 0.003 to 1.0 molar. The
metal complexes of the present invention may be
employed for visualization of various sites. For
example, for the visualization of brain lesions using
15   magnetic resonance imaging, a gadolinium complex of a
ligand of the invention, including a ligand of the
formula I or formula II, may be administered
intravenously at a dose of 0.001 to 0.5 millimoles of
the complex per kilogram of body weight, preferably
20   at a dose of 0.001 to 0.3 millimoles/kilogram. For
visualization of the kidneys, the dose is preferably
0.05 to 0.20 millimoles/kilogram. For visualization
of the heart, the dose is preferably 0.001 to 0.3
millimoles/kilogram. For visualization of the liver,
25   the dose is preferably 0.001 to 0.3
millimole/kilogram.

      The pH of the formulation of the present metal
complexes is preferably between about 6.0 and 8.0,
most preferably between about 6.5 and 7.5.
30   Physiologically acceptable buffers (e.g.,
tris(hydroxymethyl)-aminomethane) and other
physiologically acceptable additives (e.g.,
stabilizers such as parabens) may also be present.

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It is also advantageous to employ dual
scavenging excipients such as those described in
copending application U.S. Serial No. 032,763, filed
March 15, 1993, entitled "DUAL FUNCTIONING EXCIPIENT
5   FOR METAL CHELATE CONTRAST AGENTS", incorporated
herein by reference. Those excipients have a general
formula corresponding to:

$$D_s[D'(L')]_t$$
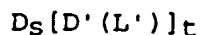
10   wherein D and D' are independently Ca or Zn, L' is an
organic ligand which may be different from, or the
same as, the ligand employed to complex the metal,
and s and t are independently 1, 2 or 3.
        As already noted, the present invention
15   further includes multimeric forms of the compounds of
the invention, including those of formula I and
formula II, such as dimers, trimers, tetramers, etc.
Known functional groups and technology are readily
useable to provide such multimers.
20   Compounds of the present invention may include
those containing functional group(s) capable of
forming a conjugate with a biomolecule. These
compounds are preferably chelates, including a
functional group, capable of exhibiting an
25   immobilized relaxivity between about 60 and 200
$mM^{-1}s^{-1}$/metal atom, for example between about 70 and
150 $mM^{-1}s^{-1}$/metal atom, or between about 80 and 100
$mM^{-1}s^{-1}$/metal atom. Similarly, the chelates, once
conjugated to a biomolecule of size greater than or
30   equal to about 40,000 daltons, are also preferably
capable of exhibiting a relaxivity between about 60
and 200 $mM^{-1}s^{-1}$/metal atom, for example between about
70 and 150 $mM^{-1}s^{-1}$/metal atom, or between about 80 and
100 $mM^{-1}s^{-1}$/metal atom. Preferred biomolecules are

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peptides, polypeptides and oligosaccharides or
fragments thereof, although other biomolecules such
as proteins, particularly monoclonal antibodies,
lipids, sugars, alcohols, bile acids, fatty acids,
5 receptor-binding ligands, amino acids and RNA, DNA or
modified fragments of these may be conjugated to the
compounds of the present invention. For smaller
biomolecules, the enhanced relaxivity afforded by the
chelates of this invention may be more fully realized
10 when the chelate-biomolecule conjugate becomes
immobilized in vivo, such as by binding to a receptor
on a cell surface or by binding to another
biomolecule.

Conjugates where a compound of the invention,
15 or salt and/or multimer thereof, is linked to a
biomolecule such as a protein, provided by the
present invention, are novel, as are metal complexes
and pharmaceutical compositions containing, and
methods of using (e.g., for imaging), the
20 aforementioned conjugates. Conjugation may be
achieved in vitro, such as by use of known
conjugation methodologies, or in situ in a living
subject by administration of a compound containing
one or more of the aforementioned functional groups.
25 For linking the compounds of the present
invention to a protein, the R groups may be reacted
with a protein to produce a protein conjugate.
Preferred proteins are those in serum, wherein the
compound of the invention is directly injected and
30 the conjugate is formed in situ. It is understood
that other functional groups, as described above, may
be used to link the bifunctional metal-chelating
ligands of this invention to proteins such as
monoclonal antibodies or fragments thereof.

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        Compounds of the formula I can generally be
prepared as follows:
        Ligands in which the aza macrocyclic ring
carbon atoms are modified are built de novo from
5 suitable aziridine precursors by
cyclotetramerization.
        Cyclotetramerization of N-benzylaziridine has
been reported in the literature. (See, for example,
T. E. Burg and G. R. Hansen, J. Heterocyclic
10 Chemistry, (1968), 5, 305.) The synthetic approach
to the preparation of S,S,S,S-tetramethyltetra-
azacyclododecane (5) is given in scheme 1:

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SCHEME 1
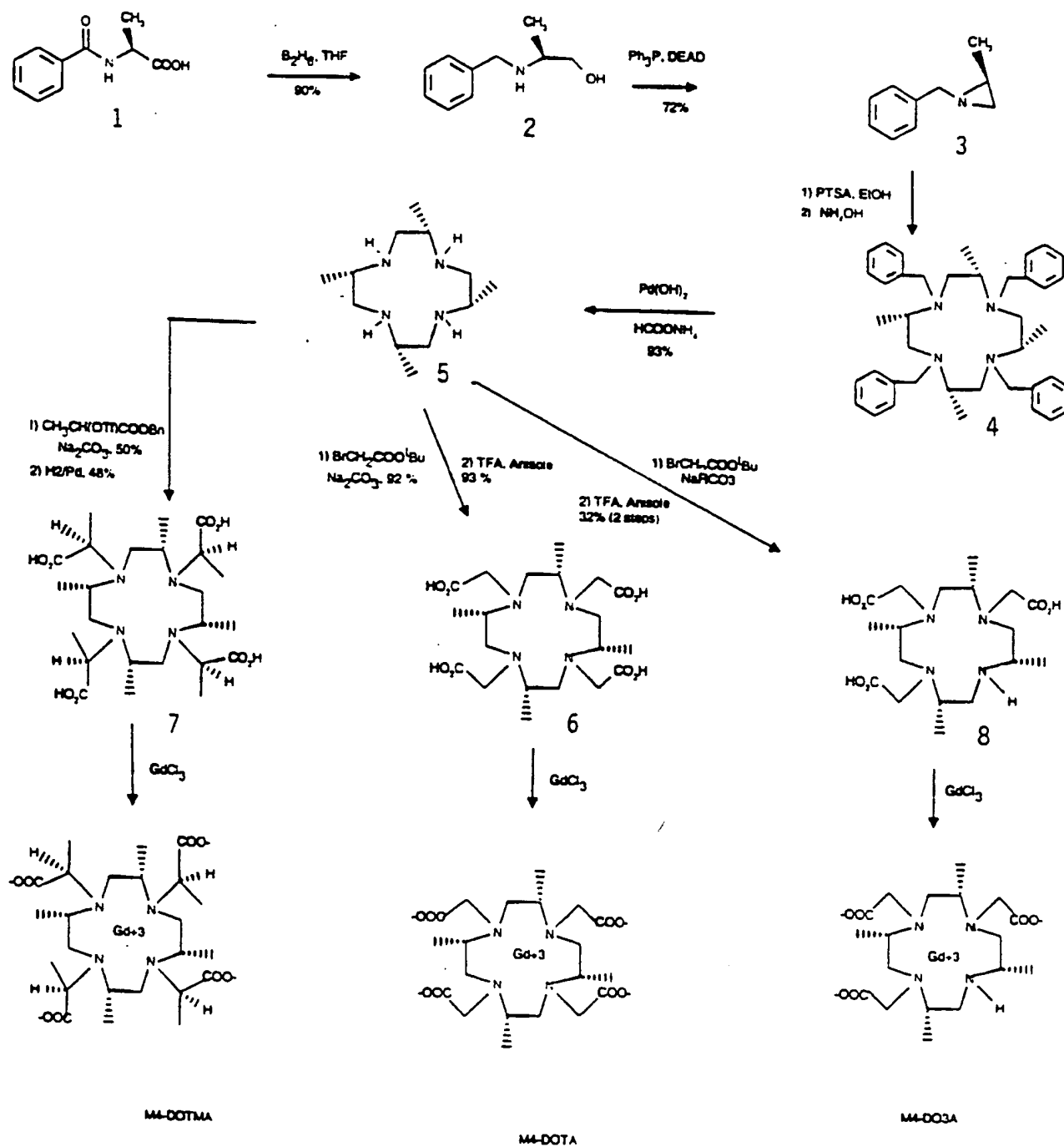
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[S]-N-Benzoylalanine(1) is reduced with
diborane to yield [S]-N-benzylalaninol(2). Under
Mitsunobu conditions, compound (2) affords [S]-N-
benzyl-2-methyl aziridine(3). Cyclotetramerization
5   under p-toluene sulfonic acid catalysis in ethanol,
followed by treatment with ammonium hydroxide
(NH₄OH), furnishes S,S,S,S-tetra-N-benzyl-
tetramethyltetraazacyclododecane(4), which is
debenzylated under transfer hydrogenolytic conditions
10   to obtain S,S,S,S-tetramethyltetraaza-
cyclododecane(5).

Tetraalkylation of compound (5) with t-butyl
bromoacetate in the presence of sodium carbonate,
followed by deprotection with trifluoroacetic acid
15   and anisole, affords the ligand (6).

Tetraalkylation of compound (5) with
benzyl 2-triflyloxylactate (prepared as described by
S.I. Kang et al., Inorg. Chem., (1993), 32, 2912-
2918) in the presence of sodium carbonate, followed
20   by catalytic hydrogenolytic debenzylation furnishes
the ligand (7).

Tris-alkylation of compound (5) with t-butyl
bromoacetate in the presence of sodium bicarbonate,
followed by deprotection by treatment with
25   trifluoroacetic acid and anisole, provides the ligand
(8).

For preparing multimeric ligands, 1,4,7-tris-
carboxymethyl-1,4,7,10-tetraazacyclododecane-10-yl
(DO3A) units, for example, are attached to a bridging
30   unit by two different methods. In the first method,
a spiro epoxide on the bridging unit is used to
alkylate DO3A. In the second method, a glycidyloxy
moiety is attached to the bridging unit which then is
used to alkylate DO3A.

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By way of example, N-alkylation of DO3A with
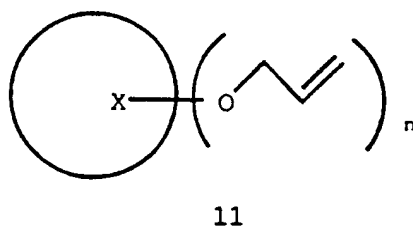suitable epoxides of formula (9) below, wherein X is
a carbocyclic, fused heterocyclic or spiro-
heterocyclic unit, and n is 2, 4 or 8, affords
5   multimeric ligands of the formula (10):

          9   10
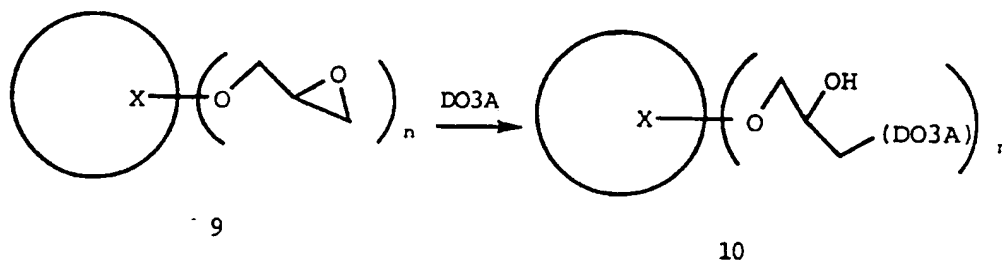
Epoxides of formula (9) are generated by the
10 Prilezhaev Reaction in which olefins of formula (11)
are treated with peracids such as m-chloroperbenzoic
acid:

15   11
Olefins of formula (11) are generated by the
alkylation of alcohols of formula (12):

                               12

20
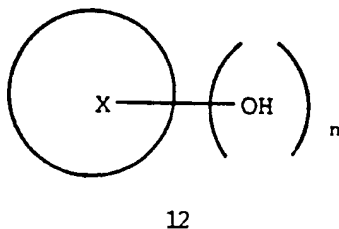
Another mode of attachment of DO3A would be to
epoxidize olefins of formula (13) to obtain epoxides

of formula (14), and then alkylating DO3A with the
epoxides to form ligands of formula (15):
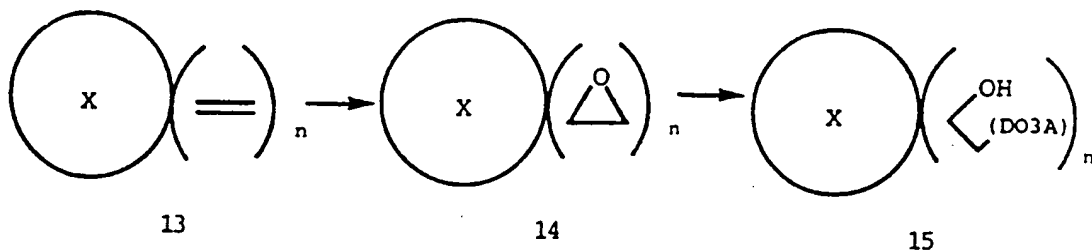
5

These two method of attachment of DO3A could
also be present in the same bridging unit X leading
to structures as in formula (16):
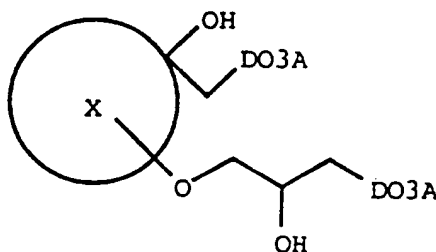
10   16

Representative bridging units are exemplified
in formulas (17), (18), (19), (20), (21) and (22):

15   17   18   19
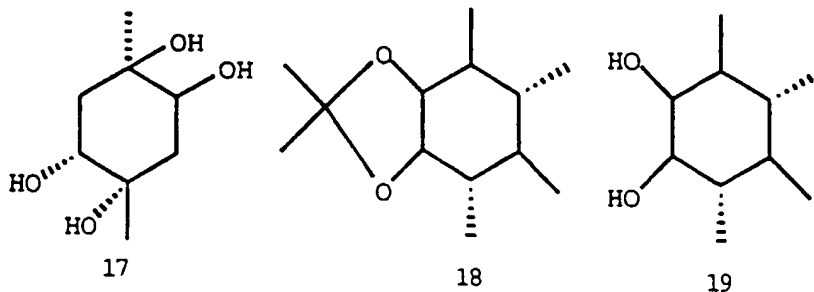
- 24 -
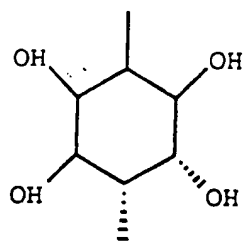
20
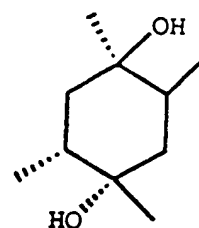
21
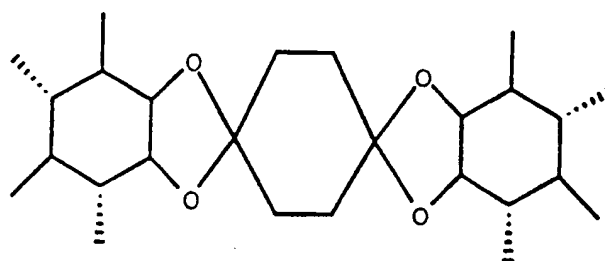
22

The structures shown in formulas (17) to (22)
5 are only by way of representative examples. They
will, in actuality, be a mixture of all the possible
diastereomers, if the attachment is through a 2-
hydroxypropyloxy link. In the case of formula 22,
for example, in addition to the presence of
10 diastereomers, the product would also consist of the
various geometric isomers that could result when the
two myo-inositol molecules are coupled to each other.
The coupling of the two myo-inositol units is
achieved by reacting the compound of formula (23)
15 with cyclohexane-1,4-dione (24):

- 25 -

24   23
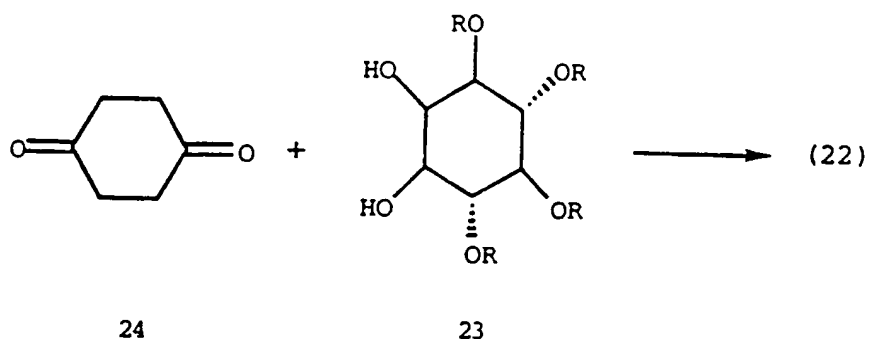
The bridging unit for formula 17 is generated
from commercially available diethyl cyclohexane-1,4-
dione-2,5-dicarboxylate by lithium aluminum hydride
reduction as described in J.G. Murphy, *J. Med. Chem.*,
(1966), *2*, 157.

The bridging unit for formula (18) is
generated by treating myo-inositol (25) with 2,2-
dimethoxypropane in the presence of p-toluene
sulfonic acid as descirbed by Giggs et al.,
Carbohydrate Res., 1985, *142*, 132:
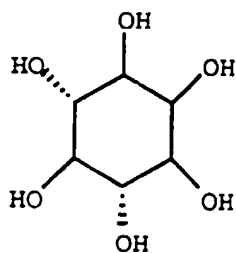
25

The bridging unit (19) is made from (18) by
acidic hydrolysis. The unit (20) is prepared from
myo-inositol (25) by reaction with excess 2,2-
dimethoxypropane in the presence of p-toluene-
sulfonic acid. The unit (21) is made from the bis-

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epoxide precursor to the unit (17) by methods
desribed above for functionalizing hydroxy groups.

The ligand of Example (10), below, bearing a
phosphonomethyl arm, is made by treating DO3A with
5   phosphorus acid and formaldehyde as described by M.
Tazaki et al., Chem. Lett., 1982, 571.

All stereoisomers of the compounds and
complexes of the present invention are contemplated
herein, whether alone (that is substantially free of
10   other isomers), in a mixture of certain stereoisomers
(for example, as a racemate) or in any other mixture
thereof.

The invention will now be further described by
the following examples. In the structures of
15   examples 1 - 6, R is
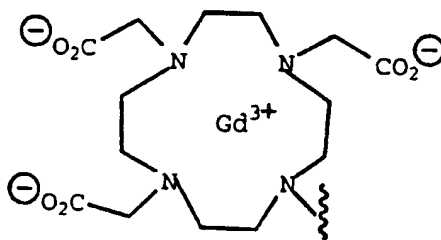
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Example 1

(1α,2α,4ß,5ß)-10,10'-[(1,2,4,5-Tetrahydroxy-
1,4-cyclohexanediyl)bis(methylene)]bis[1,4,7,10-
5   tetraazacyclododecane-1,4,7-triacetic acid],
digadolinium salt
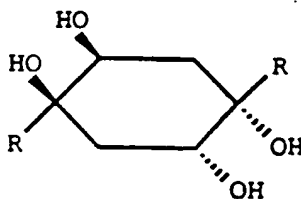
10   A.   trans-2,5-dihydroxy-1,4-dimethylene cyclohexane
             A warm solution of dry, recrystallized diethyl
     1,4-cyclohexanedione-2,5-dicarboxylate (30.75 g, 120
     mmol) in 350 ml anhydrous tetrahydrofuran (THF) was
     added to a refluxing solution of 1.0 M LAH in THF
15   (600 mL) over a 2 hour period. After an additional
     1.5 hours at reflux and subsequent cooling, a
     saturated solution of aqueous Rochelles's salt (app.
     73 mL) was added dropwise to the reaction mixture.
     The tartrate complex was filtered, washed with THF
20   and the filtrate was concentrated to a paste. The
     paste was recrystallized from hot ethyl acetate;
     yield 4.39 g (31.3 mmol, 26.1%). A second
     recrystallization yielded 3.81 g (27.2 mmol, 22.6%).
     M.P.: 160 - 161.5°C, uncorrected (lit. 162.5 -
25   163°C).

     B.   (3α,4α,6β,9β)-1,7-dioxadispiro[2.2.2.2]-
             decane-4,9-diol
             A solution of wet, 80% m-chloroperoxybenzoic
30   acid (mCPBA, 7.00 g, app. 30 mmol) in 30 mL dry
     dichloromethane (CH$_2$Cl$_2$) was dried over anhydrous

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magnesium sulfate (MgSO4). This solution was added
to a solution of dry Compound A (1.40 g, 10.0 mmol)
in 15 mL dry CH2Cl2). The mixture was stirred at
room temperature under a dry nitrogen atmosphere for

5   16 hours. A second sample (550 mg) of mCPBA was
added to ensure complete conversion of the
dimethylene compound to the diepoxide. After 3
hours, the mixture was evaporated to dryness and
excess mCPBA/m-chlorobenzoic acid was removed by

10 treating the residue with diethyl ether. The
ether-insoluble material was filtered, washed with
ethyl ether (Et2O) and air dried to yield 1.35 g of
crude Compound B. The crude material was
recrystallized from a minimum of hot methanol; yield

15 967 mg (5.6 mmol, 56.1%).
    M.P.: 231 - 233°C.

    C.   (1α,2α,4β,5β)-10,10'-[(1,2,4,5-Tetrahydroxy
            -1,4-cyclohexanediyl)bis(methylene)]
20   bis[1,4,7,10-tetraazacyclododecane-1,4,7-
            triacetic acid], hexasodium salt
    The pH of a suspension of 1,4,7,10-tetra-
    azacyclododecane-1,4,7-triacetic acid (DO3A)-H2SO4
    (prepared as described in D.D. Dischino et al.,

25 Inorg. Chem., 1991, 30, 1265 - 69; 2.67 g, 6.0 mmol)
    in 2.0 mL water was adjusted to approximately pH 12.5
    with 5.40 mL 5N sodium hydroxide. This solution was
    warmed to 50°C, and Compound B (517 mg, 3.0 mmol) was
    added portion-wise over 5.5 hours. Crystals

30 developed overnight from the warm mixture. After
    cooling, the crude crystals (3.4 g) were isolated and
    were recrystallized from 20 mL hot water to yield 1.3
    g (1.4 mmol, 45%) of Compound C.

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Mass Spectrum (FAB): (M-7H+8Na)$^+$ at 1040; (M-6H+7Na)$^+$ at 1019; (M-5H+6Na)$^+$ at 997; (M-4H+5Na)$^+$ at 975; (M-3H+4Na)$^+$ at 953; (M-2H+3Na)$^+$ at 931.
Elemental analysis: Found: C 42.53, H 6.10, N
5   10.90. Calculated for: $C_{36}H_{58}N_8Na_6O_{16} \cdot 1.16$ H_2O: C 42.49, H 5.97, N 11.01.

D.   ($1\alpha, 2\alpha, 4\beta, 5\beta$)-10,10'-[1,2,4,5-Tetrahydroxy
        -1,4-cyclcohexanediyl)bis(methylene)]
10   bis[1,4,7,10-tetraazacyclododecane-1,4,7-
        triacetic acid],digadolinium salt

        The pH of a solution of Compound C (760 mg,
762 µmol) in 5.0 mL deionized water was adjusted to
pH 4.83 with 225 µL acetic acid (HOAc). A solution
15   of Gd(OAc)$_3 \cdot 4$ H_2O (680 mg, 1.67 mmol) in 5.0 mL warm
deionized water was added to the ligand solution over
a 10 minute period; final pH 4.73. After 1 minute of
stirring at room temperature, a precipitate began to
develop. The volume of the precipitate increased
20   with time and the reaction was left to run overnight.
The precipitate was filtered, extensively washed with
water, and dried; yield 741 mg (631 µmol, 83%). An
analytical sample was prepared by recrystallization
from hot water.
25   Mass spectrum: (FAB): (M+H)$^+$ at 1169 through 1177.
Elemental analysis:
Found: C 35.93, H 5.50, N 8.84.
Calculated for $C_{36}H_{58}Gd_2N_8O_{16} \cdot 2.5$ H_2O: C 35.49,
H 5.21, N 9.20.

30

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Example 2

(3aα,4α,5β,6α,7β,7aα)-10,10·10·,10·'-[[Hexahydro-2,2-
dimethyl-1,3-benzodioxol-4,5,6,7-tetrayl]tetra(oxy)-
tetra(2-hydroxy-3,1-propanediyl)]tetrakis[1,4,7,10-
tetraazacyclododecane-1,4,7-triacetic acid],
tetragadolinium salt
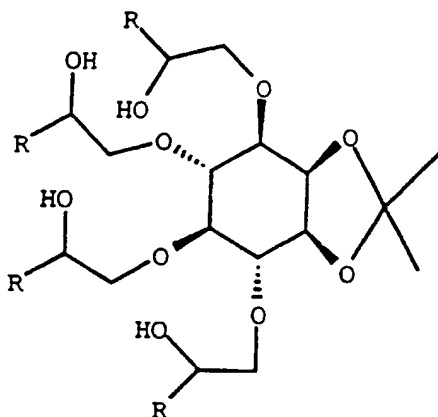
A.   (+)-1,2-O-Isopropylidene-myo-inositol
         A mixture of myo-inositol (25.0 g, 140 mmol),
2,2-dimethoxypropane (42.5 mL, 350 mmol) and p-
toluenesulfonic acid (250 mg) in dimethylsulfoxide
(80 mL) was stirred at 100°C for 45 minutes until the
solution became homogenous. After stirring the
mixture at ambient temperature for 30 minutes,
triethylamine (2.5 mL), ethanol (100 mL) and ether
(500 mL) were introduced causing precipitation. The
solid material was filtered, washed with ether-
methanol (200 mL, 5:1) and ether, and dried to obtain
the ketal compound A (9.72 g) as a white solid in 32%
yield.
       m.p.:   182.4 - 182.9°C

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B.   (3aα,4α,5ß,6α,7aα,)-Hexahydro-2,2-
        dimethyl-tetra-kis-4,5,6,7-(2-propenyl-
        oxy)-1,3-benzodioxole

        To a suspension of sodium hydride (545 mmol)
5   in dimethylformamide (50 mL) was added the ketal
    compound A (20.0 g, 90.8 mmol) dimethylformamide (20
    mL) at ambient temperature under nitrogen. After
    stirring the suspension for 2 hours, allyl bromide
    (83.4 g, 689 mmol) in dimethylformamide (50 mL) was
10 reacted for 18 hours. The unreacted sodium hydride
    was carefully quenched with water, and then the
    solvent was removed to obtain a dark brown oil. The
    residue was partitioned in water (100 mL) and
    dichloromethane (100 mL) and extracted with
15 dichloromethane (100 mL x 3). The combined organic
    layers were dried over sodium bisulfate and
    evaporated to obtain a brownish liquid (about 45 mL).
    The crude product was purified by silica gel flash
    chromatography (ethyl acetate and hexanes, 1:8) to
20 afford compound B (26.2 g) as a pale yellow liquid in
    76% yield.

C.   (3aα,4α,5ß,6α,7aα,)-Hexahydro-2,2-
        dimethyl-tetra-kis-4,5,6,7-(oxiranylmethoxy)-
25   1,3-benzodioxole

        To compound B (6.0 g, 15.8 mmol) in
    dichloromethane (10 mL) was added dropwise m-
    chloroperbenzoic acid (85% from ICN, 19.2 g, 94.6
    mmol) dissolved in dichloromethane (100 mL). A
30 homogeneous solution was initially obtained but a
    white solid, presumed to be m-chlorobenzoic acid,
    slowly precipitated out of the solution after about
    15 minutes. After 24 hours, sodium metabisulfite
    (100 mL, 5% in water) and sodium bicarbonate (200 mL,

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10% in water) were added and the resulting solution
was extracted with dichloromethane (100 mL x 3). The
organic layers were combined and dried over sodium
sulfate. The residue, obtained from removal of the
5   solvent, was purified by flash silica gel
chromatography to obtain compound C as a colorless
liquid in 76% yield.

D.   (3aα,4α,5ß,6α,7ß,7aα,)-
10   10,10',10'',10'''-[[Hexahydro-2,2-dimethyl-
1,3-benzodioxol-4,5,6,7-tetrayl]-
tetra(oxy)tetra(2-hydroxy-3,1-
propanediyl)]tetrakis[1,4,7,10-tetraazacyclo-
dodecane-1,4,7-triacetic acid], triethylamine
15   (1:4) salt

To a suspension of DO3A (9.77 g, 28.2 mmol)
dissolved in alkaline water (6 mL, 10 N sodium
hydroxide was used to obtain a pH 10 solution) at 40-
50°C was added the tetraepoxide compound C (1.54 g,
20 3.46 mmol) in acetonitrile (5 mL). The reaction
mixture was stirred for 2 days maintaining the same
pH. The crude product was purified by DEAE Sephadex
chromatography using triethylammonium bicarbonate (pH
7.5) as the eluent. The buffer concentration to
25 bring out the product compound D was 200 to 500 mM.
Removal of the buffer gave compound D (7.8 g) in 58%
yield.
m.p.:   178°C (decomp.)

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E.   (3aα,4α,5β,6α,7β,7aα)-10,10'10",10·'-
[[Hexahydro-2,2-dimethyl-1,3-benzodioxol-
4,5,6,7-tetrayl]tetra(oxy)tetra(2-hydroxy-
3,1-propanediyl)]tetrakis[1,4,7,10-
tetraazacyclododecane-1,4,7-triacetic acid],
<u>tetragadolinium salt</u>

To compound D (216 mg, 96 μmol) dissolved in water (1 mL) was added gadolinium acetate (236 mg, 580 μmol) dissolved in water (2 mL). The solution was heated to 65°C for 18 hours. The pH was maintained between 4.0 and 6.0 during the chelation reaction. The material was loaded to a CHP20P column and eluted with water (1.9 L) followed by 10% ethanolic water (1 L). The product was eluted with 10% ethanol-containing fraction, as identified by HPLC, and evaporated to dryness to yield the title compound (283 mg) as a white crystalline solid in a quantitative yield.

MS (FAB): m/z: 2447.8 [(M+H)$^+$, base peak]. Analysis Calculated for $C_{77}H_{124}N_{16}O_{34}Gd_4$·11.41$H_2O$: C, 34.87; H, 5.58; N, 8.45.
Found: C, 34.68; H, 5.98; N, 8.30; H_2O, 7.75 (desorprtion KF).

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Example 3

3,4,5,6-Tetra-O-[2-hydroxy-3-[4,7,10-
tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-
5 yl]propyl]-myo-inositol, tetragadolinium salt
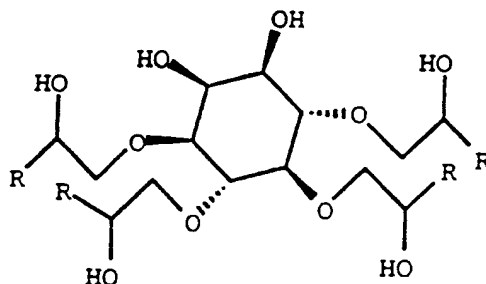
A.   3,4,5,6-Tetra-O-[2-hydroxy-3-[4,7,10-tris-
10   (carboxymethyl)-1,4,7,10-tetraazacyclo-
        dodecan-1-yl]propyl]-myo-inositol,
        tetratriethyl ammonium salt

        Compound D from Example 2 was treated with 1.0
N aqueous hydrochloric acid (10 mL) for 0.5 hours to
15 remove the ketal group. The resulting solution was
applied to a polyvinylpyridine (PVP) column (2.5 x 30
cm) and eluted with water. Silver nitrate was given
to test each fraction to detect any breakage of
chloride ion. Removal of the water from the
20 fractions containing the product gave the title
compound (910 mg) in 80% yield.
m.p.: 210°C (decomp). MS (FAB): m/z: 1789.9
[(M+H)$^+$, base peak]; 1811.9 [(M+Na)$^+$]. Analysis
Calculated for $C_{74}H_{132}N_{16}O_{34} \cdot 11.0H_2O$:
25 C, 44.63; H, 7.81; N, 11.27. Found: C, 44.63; H,
7.84; N, 11.41; H_2O, 6.43 (desorption KF); ROI, 0.17.

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B.   3,4,5,6-Tetra-O-[2-hydroxy-3-[4,7,10-
        tris(carboxymethyl)-1,4,7,10-tetraazacyclo-
        dodecan-1-yl]propyl]-myo-inositol,
        tetragadolinium salt

5   To gadolinium acetate (595 mg, 1.46 mmol)
dissolved in water (1.5 mL) was added Compound A (550
mg, 0.246 mmol) dissolved in water (2 mL). The
chelation mixture was kept overnight at 60°C and the
pH of the solution maintained between 4.0 and 5.0.
10 The resulting mixture was loaded to a CHP20P column
(2.5 x 25 cm) and eluted with water followed by 10%
ethanol. The title compound was brought out by 10%
ethanolic water. Removal of the solvents from the
fractions containing the product gave the title
15 compound as a white solid in 93% yield.
MS (FAB): 2408.7[(M+H)$^+$, ^{158}Gd)]: Analysis Calculated
for $C_{74}H_{120}N_{16}O_{34}Gd_4 \cdot 4.86$ H2O: C, 35.63; H, 5.24; N,
8.98. Found: C, 35.48; H, 5.74; N, 8.75; 3.51 H2O
(desorption KF); ROI, 31.52.
20

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Example 4

(1α,2α,3α,4β,5α,6β)-10,10'-[(2,3,5,6-tetrahydroxy-
1,4-cyclohexanediyl)bis(2-hydroxy-3,1-propanediyl)]-
5   bis[1,4,7-10-tetraazacyclododecane-1,4,7-triacetic
acid], digadolinium salt
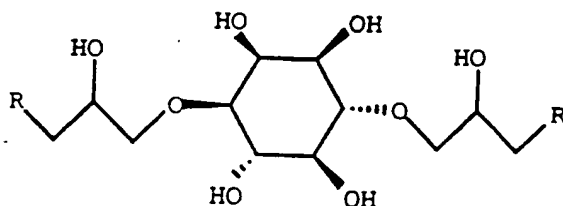
10   A.   [3aR-(3aα,4α,4aα,7aα,8β,8b)]-Hexahydro-
            2,2,6,6-tetramethyl-4,8-bis-(2-propenyl-
            oxy)benzo[1,2-d:4,5-d']bis[1,3]dioxole

       A sample of sodium hydride (NaH) (8.31 g, 156
mmol; 45% in mineral oil) was added to an ice cold
15   solution of dry 1,2:4,5-di-O-isopropylidene-
myo-inositol (10.00 g, 38.4 mmol, prepared as
indicated in Gigg et al., Carbohyd. Res., 1985, 142,
132) in dry dimethylformamide (DMF) (80 ml). After
stirring for 0.5 hours at room temperature, allyl
20   bromide (8.31 mL, 96 mmol) was added dropwise over 5
minutes. During the addition, the temperature was
maintained at 25°C by cooling in a cold water bath.
After 1.5 hours, the reaction was stopped by the
careful addition of water and the mixture was
25   evaporated to dryness in vacuo. The residue was
dissolved in water, extracted with ethyl acetate
(EtOAc) (4 x 50 ml), and washed with water (2 x 20
ml) and brine (1 x 20 ml). The organic layer was
dried over sodium sulfate (Na2SO4) and evaporated to
30   dryness under reduced pressure. The residue was

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recrystallized from hexane to give pure product as a
colorless solid (12.88 g, yield 98.5%).
M.P. 82 - 83°C, uncorrected.

5   B.   (3aα,4α,4aα,7aα,8β,8b)-Hexahydro-2,2,6,6-
              tetramethyl-4,8-bis(oxiranylmethoxy)benzo
              [1,2-d:4,5-d']bis[1,3]dioxole

     A solution of 80% mCPBA (13.09 g, 60.7 mmol)
     in dry dichloromethane (CH2Cl2) (95 ml) was added to
10   a cooled solution containing Compound A (5.11 g, 15.0
     mmol) in dry CH2Cl2 (15 ml). After stirring at room
     temperature overnight, the mixture was treated with
     saturated aqueous sodium sulfite (Na2SO3) followed by
     saturated aqueous sodium bicarbonate (NaHCO3) until
15   the aqueous layer was neutral. The organic layer was
     dried over sodium sulfate (Na2SO4) followed by
     evaporation to dryness in vacuo. The residue was
     recrystallized from hot ethanol to give the pure
     product (4.05 g, yield 72.4%).
20   M.P. 103 - 105°C (uncorrected)

     C.   (1α,2α,3α,4β,5α,6β)-10,10'-[(2,3,5,6-tetra-
              hydroxy-1,4-cyclohexanediyl)bis(2-hydroxy-3,1-
              propanediyl)]bis[1,4,7-10-tetraazacyclodode-
25   cane-1,4,7-triacetic acid], triethylamine
              (1:2) salt

     A sample of crude (3aα,5α,5β,6α,7β,7aα)-
     10-10'-[[hexahydro-2,2-dimethylbenzol[1,2-d:4,5-d']-
     bis[1,3]dioxol-4,8-diyl]di(oxy)di(2-hydroxy-3,1-
30   propanediyl)]bis[1,4,7,10-tetraazacyclododecane-
     1,4,7-triacetic acid] was prepared by heating a
     solution of DO3A·H2SO4 (28.24 g, 63.5 mmol) and
     Compound B (5.92 g, 15.9 mmol) in 10 M sodium
     hydroxide (NaOH) (28.0 mL), H2O (20 mL) and

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1,4-dioxane (17 mL) at 50°C for 70 hours. The pH of
the crude mixture was adjusted to pH 1 using 1N
hydrochloric acid (HCl) and was heated at 55°C for
1.5 hours. After the hydrolysis was complete, the pH

5 of the solution was adjusted to 9 with dilute NaOH
and the mixture was applied to a DEAE-Sephadex A-25
column (HCO3⁻ form, 2L). The column was washed with
water, and the product was eluted with a linear
gradient of 5 - 250 mM TEAB buffer (pH 7.5, 4 L

10 each). The fractions which contained pure Compound C
were combined, freed of TEAB, and lyophilized to give
Compound C as the di(triethylammonium) salt (18.22 g,
yield 97.2%). The mass spectral analysis of this
product indicated the presence of chloride ion. A

15 sample of the contaminated product (10.00 g) was
dissolved in water and was applied onto a AG50W x 8
column (H⁺ form, 500 ml). The column was washed with
water, and the product was eluted with 0.5 M ammonium
hydroxide (NH4OH). The fractions which contained

20 Compound C were combined and evaporated to give the
ammonium salt (2.91 g). The ammonium salt was
dissolved in water, applied to a DEAE-Sephadex A-25
column (HCO3⁻ form, 2L). The column was washed with
water and the product was eluted with 200 mM TEAB (pH

25 7.5, 5L). The fractions containing pure material
were combined, freed of TEAB, and lyophilized to give
pure Compound C as the di(triethylammonium) salt
(2.86 g, theo. yeild 25.9%).

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D. $(1\alpha,2\alpha,3\alpha,4\beta,5\alpha,6\beta)$-10,10'-[(2,3,5,6-
       tetrahydroxy-1,4-cyclohexanediyl)]bis(2-
       hydroxy-3,1-propanediyl)]bis[1,4,7,10-
       tetraazacyclododecane-1,4,7-triacetic
5   <u>acid], digadolinium salt</u>

       A sample of Compound C (1.02 g) was dissolved
in a solution containing Gd(OAc)$_3\cdot$4 H$_2$O (1.219 g, 3
mmol) in water (10 mL) and was heated at 50°C for 16
hours. The solution was applied onto a CHP20-P
10   column (500 mL). The salt contaminants were eluted
with water, and the product was eluted with 10%
ethanol in water. The appropriate fractions were
combined and evaporated to dryness *in vacuo*. The
glassy residue was dissolved in water, and
15   lyophilized to give pure title compound (1.04 g,
yield 80.5%).
MS (FAB): (M + H)$^+$ at 1295.2 (major isotope).
Elemental analysis:
Found: C, 36.83; H, 5.63; N, 8.46%.
20   Calculated for C$_{40}$H$_{66}$Gd$_2$N$_8$O$_{20}\cdot$1.20 H$_2$O:
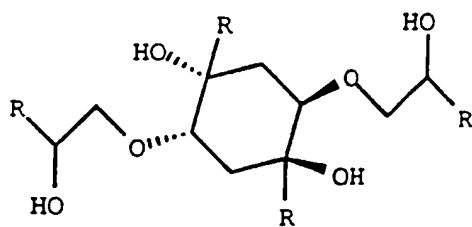   C, 36.53; H, 5.24; N, 8.52%.

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Example 5

10-[[1,4-Dihydroxy-2,5-bis[2-hydroxy-3-[4,7,10-tris-
(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-
propoxy]-4-[[4,7,10-tris(carboxymethyl)-1,4,7,10-
tetraazacyclododecan-1-yl]methyl]cyclohexyl]methyl]-
1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid,
tetragadolinium salt

A.   1,7-dioxadispiro [2,2,2,2]-decane-4,9-
       diallyldiol

     To a suspension of sodium hydride (432 mg, 18
mmol) in anhydrous dimethylformamide (15 ml), a
solution of bisepoxydiol (1.0 g, 6 mmol) (described
in Example 1B) in anhydrous dimethylformamide (60 ml)
was added dropwise and the mixture was stirred at
room temperature for 60 minutes. Allyl bromide
(2.178 g, 18 mmol) was added and the mixture was
stirred at room temperature for 18 hours. Excess of
sodium hydride was decomposed with water (2 ml),
dimethylformamide removed in vacuo and the residue
extracted with ethyl acetate (2 x 100 ml), washed
with water (2 x 50 ml) and dried. Solvent removal
afforded the crude product. Silica gel (25 g) column
chromatography using hexanes and ethyl acetate (2/1)
as the eluent afforded the pure diallyl derivative
compound A as a colorless solid (0.7 g, yield 48%).
Melting point: 63.5-65.5°C (uncorrected).

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B.   (3α,4α,6ß,9ß)-4,9-bis(oxiranylmethoxy)-
       1,7-dioxadispiro[2.2.2.2]decane

        A solution of m-chloroperoxybenzoic acid (4.3
       g, 80%, 18.5 mmol) in dichloromethane (30 ml) was
5   dried over anhydrous magnesium sulfate. This
       solution was added dropwise to a solution of compound
       A (1.25 g, 5 mmol) in dry dichloromethane (25 ml).
       The reaction mixture was stirred at room temperature
       for 20 hours. Excess of m-chloroperoxybenzoic acid
10   was decomposed with saturated aqueous sodium
       metabisulfate solution (15 ml). The organic layer
       was separated, washed with saturated sodium
       bicarbonate (2 x 50 ml), and with water (2 x 50 ml).
       The organic layer was dried over magnesium sulfate
15   and evaporated to dryness. Recrystallization of the
       crude material from hot ether afforded the pure
       tetraepoxide compound B as white crystals (1.1 g,
       yield 77.5%).
       Melting point: 93.0-96.0°C (uncorrected).
20

       C.   10-[[1,4-Dihydroxy-2,5-bis[2-hydroxy-3-
              [4,7,10-tris-(carboxymethyl)-1,4,7-10-
              tetraazacyclododecan-1-yl]-propoxy]-4-
              [[4,7,10-tris(carboxymethyl)-1,4,7,10-
25   tetraazacyclododecan-1-
              yl]methyl]cyclohexyl]methyl]-1,4,7,10-
              tetraazacyclododecane-1,4,7-triacetic acid,
              triethylamine (1:4) salt

        A solution of DO3A (17.3 g, 50 mmol) in water
30   was made (80 ml) and the pH of the solution adjusted
       to 12 with 5 N sodium hydroxide solution. The
       solution was heated to 80°C and a solution of
       compound B (1.42 g, 5 mmol) in dioxane (10 ml) was
       added dropwise. The mixture was stirred at this

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temperature for 82 hours. At the end of 82 hours, the pH of the reaction mixture was adjusted to 7 with acetic acid. The mixture was diluted and loaded on to a Sephadex G-25 column. The column was eluted

5   first with 100-250 mM triethyl ammonium bicarbonate (TEAB) buffer and then with 250-500 mM TEAB buffer. Fractions containing the pure tetrameric material were combined and solvent removal afforded the pure product as the tetra triethyl ammonium salt of the

10   title compound as a colorless glassy solid (2.85 g, yield 35%).

Mass Spectrum: 1670 $(M+H)^+$. 102 $(CH_3CH_2)_3N^+H$

Elemental Analysis: Found: C, 53.70; H, 9.15; N, 13.62; H_2O, 0.58%. Calculated for $C_{94}H_{184}N_{20}O_{30} \cdot 0.67$

15   H_2O: C, 54.11; H, 8.85; N, 13.42; O, 23.52%.

D.   10-[[1,4-Dihydroxy-2,5-bis[2-hydroxy-3-
        [4,7,10-tris-(carboxymethyl)-1,4,7,10-
        tetraazacyclododecan-1-yl]-propoxy]-4-
20   [[4,7,10-tris(carboxymethyl)-1,4,7,10-
        tetraazacyclododecan-1-
        yl]methyl]cyclohexyl]methyl]-1,4,7,10-
        tetraazacyclododecane-1,4,7-triacetic acid,
        tetragadolinium salt

25   A sample of Compound C (0.2 g) was dissolved in 3 mL deionized water (reactant I). In a separate vial, 0.18 g of $GdCl_3$ powder was dissolved in 5 mL deionized water (reactant II). At about 70°C and constant stirring, reactant I was added into reactant

30   II dropwise over a time period of several hours. Meanwhile, 5N NaOH was pipetted into the solution from time to time to neutralize the protons released from chelation and maintain the reaction pH around 6. The excess metal ions were precipitated in the form

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of M(OH)$_3 \cdot$ x H$_2$O by raising solution pH to about 9
and incubating at both about 70°C and room
temperature for several hours. The precipitate was
subsequently removed through centrifugation and
5   filtration with 0.22 μm membrane. The filtrate was
finally condensed and neutralized to pH 7 in
preparation for HPLC purification.

The crude chelate product was purified by
preparative HPLC, using mobile phase gradient
10   (acetonitrile:water) and silica-based reverse phase
column (YMC C18, 5μ, 200 A). The solvent was removed
from the fractions containing the product. The
residue was further dried in a vacuum oven at about
70°C overnight. The yield of the desired chelate was
15   70%.
Mass Spectrum (FAB, m/e):(Gd^{159} + H)$^+$ at 2287.5.
Elemental Analysis (C,H,N):
Calculated for C$_{70}$H$_{112}$N$_{16}$O$_{30}$Gd$_4 \cdot$7.73 H$_2$O: C34.66,
H 5.30, N 9.24%.
20   Found: C 34.26, H 5.36, N 9.08%.

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Example 6

[3aR-(3aα,3ˇaα,4ß,4ˇß,5α,5ˇα,6ß,6ˇb,7α,-
7ˇα,7aα,7ˇaα)]-dodecahydro-4,4ˇ,5,5ˇ,6,6ˇ,7,7ˇ-
5   octakis-[[2-hydroxy-3-[(4,7,10-tricarboxymethyl)-
1,4,7,10-tetraazacyclododecan-1-yl]propyl]-
oxy]dispiro-[1,3-benzodioxole-2,1'-cyclo-hexane-
4ˇ,2ˇ-[1,3]-benzodioxole], octagadolinium salt

10
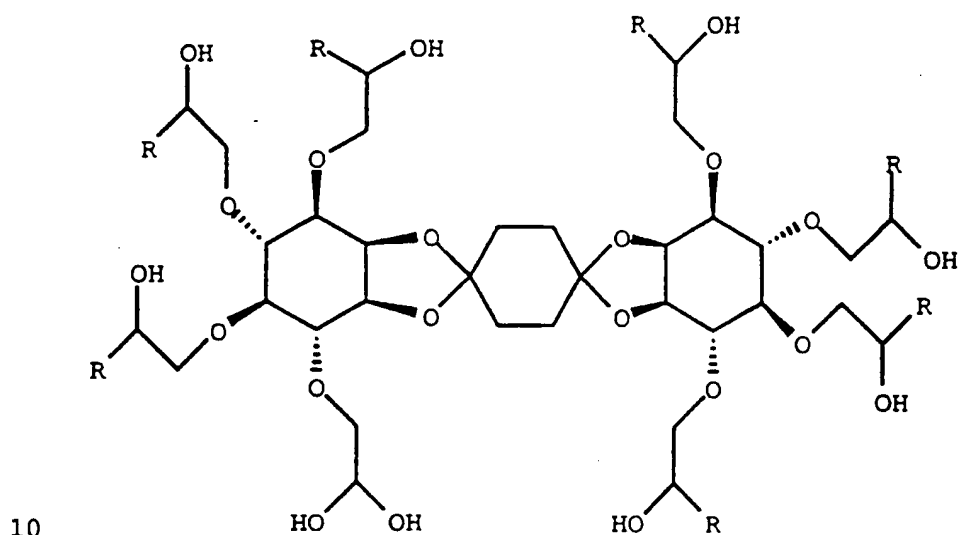
A.   (+)-3,4,5,6-Tetra-O-allyl-myo-inositol

        Compound B from Example 2 (15.3 g, 44.9 mmol)
was dissolved in methanol (30 mL) and treated with 3N
15   hydrochloric acid (30 mL), with constant stirring at
room temperature for 13 hours. The reaction mixture
was neutralized with saturated sodium bicarbonate
solution and extracted with methylene chloride (50 mL
x 3). The dichloromethane layers were combined and
20   dried with sodium sulfate, filtered, evaporated in
vacuo and purified by column chromatography (silicon
dioxide, ethyl acetate: hexanes (1:1 v/v)) to obtain
9.1 g (67%) of the desired product compound A.

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B.   [3aR-(3aα,3˙aα,4α,4˙ß,5ß,5˙α,6α,6˙ß,
        7ß,7˙α,7aα,7˙aα)]-Dodecahydro-4,4˙,5,-
        5˙,6,6˙,7,7˙-octakis-(2-propenyloxy)-
        dispiro[1,3-benzo-dioxole-2,1'-cyclo-
5   hexane-4',2˙-[1,3]-benzodioxole]

        A mixture of the diol compound A (1.0 g, 2.94
mmol), 1,4-cyclohexanedione (165 mg, 1.47 mmol) and
p-toluenesulfonic acid (55 mg, 0.289 mmol) was heated
in toluene (30 mL) at reflux for 19 hours. The crude
10   reaction mixture was purified by flash silica gel
column chromatography (about 200 g) using different
eluents of hexane and ethyl acetate (2:1, 1:1 and
1:2, 500 mL each). Three to four spots that have the
molecular ion peak at 757 (m/z) were pooled to afford
15   an oil (520 mG) in 43% yield, and the resulting
mixture used as a mixture of the isomers of the
expected octa-allyl bis-ketal product.
TLC: R_f 0.45, 0.37 and 0.32 in hexanes and : acetone
(5:1, v/v)
20

C.   [3aR-(3aα,3˙aα,4ß,4˙ß,5α,5˙α,6ß,6˙b,
        7α,7˙α,7aα,7˙aα)]-Dodecahydro-
        4,4˙,5,5˙,6,6˙,7,7˙-octakis-(oxyranylmethoxy)-
        dispiro[1,3-benzodioxole-2,1'-cyclohexane-
25   4',2˙-[1,3]-benzodioxole]

        The oxidation of the octa-allyl compound B
(1.13 g, 1.49 mmole) by m-chloroperoxybenzoic acid
(3.0 g, 85%, 17.6 mmole) in dichloromethane (20 mL)
was carried out at ambient temperature for 50 hours.
30   The white solid, which precipitated out, during the
oxidation was identified as m-chlorobenzoic acid by
^{1}H-NMR (CDCl$_3$). After removal of the solid by
filtration, the dichloromethane filtrate was treated
with sodium metabisulfite and sodium hydroxide where

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the final pH of the aqueous layer was 11.7. The
organic layer was dried over sodium sulfate and
concentrated in vacuo. The crude solid product was
purified by column chromatography (flash silica gel,
5   75 g) to obtain a white solid (670 mg) in 51% yield.

D.   [3aR-(3aα,3*aα,4ß,4*ß,5α,5*α,6ß,6*b,7α,
        7*α,7aα,7*aα)]-dodecahydro-4,4*,5,5*,-
        6,6*,7,7*-octakis-[[2-hydroxy-3-[(4,7,10-
10   tricarboxymethyl)-1,4,7,10-tetraazacyclo-
        dodecan-1-yl]propyl]oxy]dispiro-
        [1,3-benzodioxole-2,1'-cyclohexane-4',2*-
        [1,3]-benzodioxole]

The octaepoxide Compound C (200 mg, 235 μmol)
15 in acetonitrile (0.5 mL) was added to DO3A (2.41 g,
7.04 mmol) in water (4-5 mL) whose pH was adjusted to
9.6 by 10 N sodium hydroxide at 60°C. The resulting
solution was kept for 48 hours and analyzed by PRP-X
100 HPLC column at desirable intervals. Two
20 relatively pure fractions were obtained after two
DEAE Sephadex A-25 ion exchange columns. One eluted
by 300-400 mM triethyl-ammonium bicarbonate (TEAB)
showed a peak at m/z 3328.0 corresponding to the
heptamer, whereas the other by 400 mM TEAB turned out
25 to be the expected octamer with a peak at m/z 3656.6
($C_{154}H_{268}N_{32}O_{68}$).
^{1}H-NMR (D_2O): d 1.15 (t, 106H, CH_3CH_2N); 1.7-1.9 (m,
methylenes of the middle cyclohexane, 8H); 3.07 (q,
71H, CH_3CH_2N), 2.8-4.6 (m, 228H, all of methines and
30 methylenes of the octamer ligand except the
triethylamine and bridged cyclohexane). ^{13}C-NMR
(D_2O): 8.10 and 46.29 (CH_3CH_2N of triethyl-amine);
41.88, 46.29, 49.25, 49.54, 49.62, 49.76, 50.04,

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55.72, 56.43, 58.69, 172 - 175 (broad due to 24
carboxylate and/or carboxylic acid groups).
MS (FAB): 3,328 (M+H)+.
Analysis Calcualted for $C_{154}H_{268}N_{32}O_{68}$-

5    $8[N(C_2H_5)_3] \cdot 17.20H_2O$:

C, 50.81; H, 8.92; N, 11.73. Found: C, 50.33; H,
8.92; N, 11.75; H_2O, 6.10 (desorption Karl-Fisher).

E.   [3aR-(3aα,3·aα,4β,4·β,5α,5·α,6β,6·b,7α,7·α,
10   7aα,7·aα)]-dodecahydro-4,4·,5,5·,6,6·,7,7·-
         octakis[[2-hydroxy-3-[(4,7,10-tricarboxy-
         methyl)-1,4,7,10-tetraazacyclododecan-1-
         yl]propyl]oxy]dispiro-[1,3-benzodioxole-2,
         1'-cyclo-hexane-4',2·-[1,3]benzodioxole],
15   <u>octagadolinium salt</u>

Compound D (250 mg, 68.4 μmol as the octa-
triethylammonium salt) in water (5 mL) at pH 6.0 was
treated with tetrahydrated gadolinium acetate (222 mg,
546 μmol) at 60°C for 5 hours. The pH of the aqueous

20   reaction mixture was maintained at 7.0 ± 1.0. The
resulting solution was then applied to a CHP-20P
column (2.5 x 20 cm). The column was eluted with
water (750 mL), 2.5% (300 mL), 5% (300 mL) and 10%
(300 mL) of ethanol. The fractions containing the

25   desired compound, which were eluted by 10% ethanol,
were combined and concentrated in vacuo to obtain the
octameric gadolinium chelate (200 mg) as a white solid
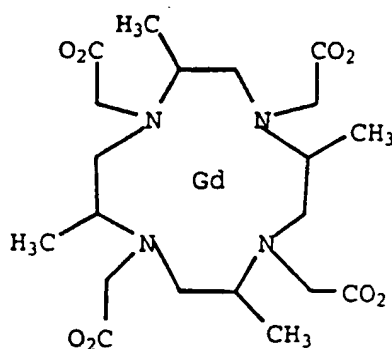in 60% yield.
Analysis Calculated for $C_{154}H_{244}N_{32}O_{68}Gd_8$ · 19.58

30   $H_2O \cdot 2.0$ C_2H_5OH: C, 35.57; H, 5.58; N, 8.40. Found: C,
35.06; H, 6.22; N, 8.42; H_2O, 6.61% (desorption
Karl-Fisher).

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Example 7

[2S-(2α,5α,8α,11α,)]-2,5,8,11-Tetramethyl-1,4,7,10-
tetraazacyclododecane-1,4,7,10-tetraacetic acid,
gadolinium salt

A.   L-2-Benzylamino propanol

To a solution of N-benzoyl-L-alanine (29.0 g,
150 mmol) in tetrahydrofuran (200 ml) at 0°C was
added a tetrahydrofuran solution of diborane (1 M
solution, 800 ml) and the mixture was refluxed for 18
hours. Excess of diborane was decomposed with
methanol and the solvents were removed under reduced
pressure. The residue was dissolved in methanol (100
ml) and treated with 6 N hydrochloric acid (100 ml).
The mixture was heated at 70°C for 12 hours and the
solvents were removed under reduced pressure. The
residue was co-evaporated with methanol (5 x 150 ml)
and the product was dissolved in water (50 ml),
basified with 5 N sodium hydroxide to pH 12 and
extracted with ethyl acetate (3 x 200 ml). The
combined organic layers were washed with saturated
sodium chloride (150 ml), dried and concentrated to a
final volume of 100 ml. 300 ml of hexane was added
and the solution cooled in the refrigerator

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overnight. The white crystalline needles deposited
were collected and dried to afford pure compound A
(41.4 g, yield 87%). m.p. 46-48°C.

5   B.   (S)-1-Benzyl-2-methyl-ethyleneimine
            To a solution of Compound A (33.0 g, 200 mmol)
and triphenyl phosphine (79.66 g, 300 mmol) in ether
(500 ml) stirred under nitrogen in an ice bath, was
slowly added diethyl azo-dicarboxylate (95%, 50 ml,
10   300 mmol). The solution was stirred at room
temperature fc - 16 hours. A crystalline precipitate
(triphenylphosphine/diethyl hydrazine carboxylate
complex) was filtered off and washed with
hexane/ether (1:1, 200 ml). The ether solution was
15   extracted with 1 N hydrochloric (2 x 100 ml). The
hydrochloric solution was basified with 5N sodium
hydroxide, extracted with ether (3 x 150 ml), dried
and concentrated to afford the crude aziridine
compound B as a yellow oily liquid. This was further
20   purified by distillation under reduced pressure to
afford pure compound B as a colorless liquid (24.3 g,
yield 75%). b.p. 71-72°C at 4 mm.

      C.   [2S-(2α,5α,8α,11α)]-2,5,8,11-Tetramethyl-
25   1,4,7,10-tetrakis(phenylmethyl)-1,4,7,10-
              tetraazacyclododecane
            To a solution of compound B (19.1 g, 130 mmol)
in ethanol (250 ml) was added p-toluenesulfonic acid
(PTSA, 1.1 g, 6.5 mmol) and the mixture was stirred
30   at room temperature for 64 hours. At the end of this
time, an additional 1.1 g (6.5 mmol) of PTSA was
added and the mixture was stirred for 48 hours.
Ethanol was removed in vacuo, and the product was
purified by column chromatography over silica gel

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(400 g) using methanol as the eluent. Fractions
containing the pure product were combined, and
solvent removal afforded 5.8 g of a salt. This was
dissolved in methanol (100 mL) and basified with
5   concentrated ammonia solution. The precipitated
solid was filtered, dried and recrystallized from
absolute ethanol to afford pure compound C as a
colorless microcrystalline solid (2.8 g, yield 14%).
m.p.:   147-148°C

10

D.   [2S-(2α,5α,8α,11α,)]-2,5,8,11-Tetramethyl-
         1,4,7,10-tetraazacyclododecane

To a solution of compound C (2.35 g, 4 mmol)
in ethyl acetate (40 mL) and ethanol (400 mL) was
15   added ammonium formate (2.52 g) and palladium acetate
on Carbon (20%, 2.35 g). The mixture was stirred
under reflux for 16 hours. The solution was filtered
to remove the catalyst and the solvents were removed
to afford pure Compound D as a light yellow solid
20   (840 mg, yield 92%).

E.   [2S-(2α,5α,8α,11α)]-2,5,8,11-Tetramethyl-
         1,4,7,10-tetraazacyclododecane-1,4,7,10-
         tetraacetic acid

25   To a solution of compound D (570 mg, 2.5 mmol)
in acetonitrile (200 mL) was added potassium
carbonate (4.0 g) and t-butyl bromo acetate (2.34 g,
12 mmol). The mixture was stirred at room
temperature for 18 hours. Potassium carbonate was
30   filtered off, acetonitrile removed in vacuo and the
residue purified by column chromatography over silica
gel (50 g) using chloroform and methanol to afford
the tetra butyl ester (1.6 g). This material was
dissolved in trifluoroacetic acid (150 mL), anisole

- 51 -

(10 mL) was added, and the mixture was stirred at
room temperature for 16 hours. Trifluoroacetic acid
was removed _in vacuo_ and anisole was removed by co-
evaporation with water (6 x 50 mL) to afford the

5   crude product. The residue was dissolved in water
(100 mL) and purified by anion exchange column
chromatography over AG1-X2 resin (150 mL). The
column, after washing with water, was eluted with 1 M
formic acid. The fractions containing the pure

10   product were combined. Solvent removal afforded the
pure product. A small amount of formic acid that
remained in the sample was removed by co-evaporation
with water (5 x 50 mL) to obtain pure title compound
as a colorless glassy solid (550 mg, yield 87%).

15

F.   [2S-(2α,5α,8α,11α,)]-2,5,8,11-Tetramethyl-
         1,4,7,10-tetraazacyclododecane-1,4,7,10-
         tetraacetic acid, gadolinium salt

         A sample of Compound E (0.1 g) was dissolved

20   in 8 mL deionized water. 0.04 mL 5N sodium hyroxide
(NaOH) was added to convert the ligand into the
monosodium salt (reactant I). In a separate vial,
0.08 g of $GdCl_3$ powder was dissolved in 1 mL
deionized water (reactant II). At about 70°C and

25   constant stirring, reactant II was added into
reactant I dropwise over a time period of several
hours. Meanwhile, 5N NaOH was pipetted into the
solution from time to time to neutralize the protons
released from chelation and maintain the reaction pH

30   around 6. The excess metal ions were precipitated in
the form of $M(OH)_3 \cdot xH_2O$ by raising solution pH to
about 9.5 and incubating at both about 70°C and room
temperature for serveral hours. The precipitate was
subsequently removed through centrifugation and

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filtration with a 0.22 μm membrane. The filtrate was
finally condensed and neutralized to pH 7 in
preparation for HPLC purification.

    The crude chelate product was purified by
5 preparative HPLC, using mobile phase gradient
($CH_3CN:H_2O$) and silica-based reversed phase column
(YMC C18, 5μ, 120 A). The solvent was removed from
fractions containing the desired product. The
residue was dried in a vacuum oven at about 70°C
10 overnight, furnishing the chelate in 65% yield.
Mass Spectrum (FAB, m/e): Gd_{159} + Na)$^+$ at 638.
Elemental Analysis (C, H, N):
Calculated for $C_{20}H_{33}N_4O_8GdNa$ • 5.01 H_2O: C, 33.00;
H, 5.96; N, 7.70%. Found: C, 32.94; H, 5.63; N, 7.75%
15

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Example 8

[αR-(aR*,α'R*,α"R*,α'''R*,2S*,5S*,8S*,11S*)]-α,-
α',α",a''',2,5,8,11-Octamethyl-1,4,7,10-

5   tetraazacyclododecane-1,4,7,10-tetraacetic acid,
gadolinium salt
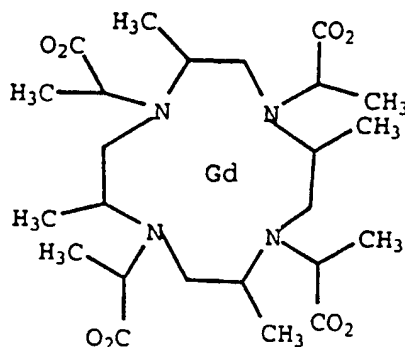
10   A.   [αR-(aR*,α'R*,α"R*,α'''R*,2S*,5S*,8S*,11S*)]-α,-
             α',α",a''',2,5,8,11-Octamethyl-1,4,7,10-
             tetraazacyclododecane-1,4,7,10-tetraacetic
             acid

             To a solution of compound D from Example 7
15   (570 mg, 2.5 mmol) in acetonitrile (200 ml) was added
     potassium carbonate followed by L-benzyl-2-
     trifluoromethanesufonyloxy-propionate (prepared fresh
     from benzyl lactate as described in S.I. Kang et al.,
     Inorganic Chem., 1993, 32, 2912 -2918) (3.9 g, 12.5
20   mmol) and the mixture was stirred at room temperature
     for 48 hours. Potassium carbonate was filtered off,
     acetonitrile removed in vacuo and the residue
     purified by column chromatography over silica gel
     (100 g) using chloroform and the methanol to afford a
25   tetra benzyl ester (1.1 g). This material was
     dissolved in a mixture of ethanol (75 ml) and water
     (10 ml) and hydrogenated over 10% Pd/C (250 mg) for

- 54 -

18 hours. The catalyst was filtered off and solvent removal afforded the crude product. This was dissolved in water (100 ml) and purified by anion exchange column chromatography over AG1-X2 resin (150 ml). The column, after washing with water, was eluted with a gradient of 0-200 mM formic acid. Fractions containing the pure product were combined. Solvent removal afforded the pure product. A small amount of formic acid that remained in the sample was removed by co-evaporation with water (5 x 50 ml) to obtain pure Compound A as a colorless glassy solid (340 mg, yield 26%).

B.   [αR—(aR*,α'R*,α*R*,α'''R*,2S*,5S*,8S*,11S*)]-α,-α',α*,a''',2,5,8,11-Octamethyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, gadolinium salt

A sample of Compound A (0.15 g) was dissolved in 8 mL deionized water. 0.056 mL 5N NaOH was added to convert the ligand into the monosodium salt (reactant I). In a separate vial, 0.11 g of $GdCl_3$ powder was dissolved in 1 mL deionized water (reactant II). Reactants I and II were treated as described in the method of Example 7F to yield 74% of the title compound.

Mass Spectrum (FAB, m/e): $(Gd^{159} + Na)^+$ at 694.

Elemental analysis: Calculated for $C_{24}H_{40}N_4O_8GdNa \cdot 1.98 H_2O$: C, 39.57, H, 6.08, N, 7.69%. Found: C, 39.31, H, 6.19, N, 7.60%.
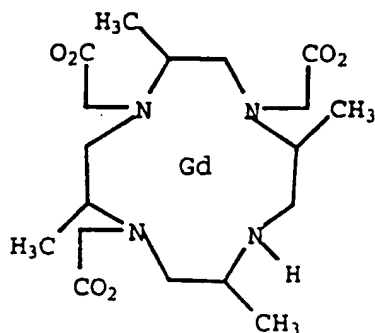
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Example 9

[2S-(2R*,5R*,8R*,11R*)]-2,5,8,11,-Tetramethyl-
1,4,7,10-tetraazacylcododecane-1,4,7-triacetic acid,
gadolinium salt

5

A.   [2S-(2R*,5R*,8R*,11R*)]-2,5,8,11-Tetramethyl-
      1,4,7,10-tetraazacyclododecane-1,4,7-triacetic
10   acid

      To a solution of Compound D (456 mg, 2 mmol)
in acetonitrile (150 mL) was added sodium bicarbonate
(4.0 g) and t-butyl bromo acetate (2.34 g, 12 mmol),
and the mixture was stirred at room temperature for
15 48 hours. Sodium bicarbonate was filtered off,
acetonitrile was removed *in vacuo* and the residue was
dissolved in TFA (25 mL). Anisole (1 mL) was added
and the mixture was stirred at room temperature for
12 hours. TFA was removed *in vacuo* and anisole was
20 removed by co-evaporation with water (5 x 50 mL) to
afford the crude product. The residue was dissolved
in water (60 mL) and purified by anion exchange
column chromatography over AG1-X2 resin (100 mL).
The column, after washing with water, was eluted with
25 a gradient of water to 50 mm M formic acid. The
fractions were analyzed by HPLC and those containing
the pure product were combined. Solvent removal
afforded Compound A. A small amount of formic acid

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that remained in the sample was removed by
co-evaporation with water (5 x 50 mL) to obtain pure
Compound E as a colorless glassy solid (320 mg, yield
40%).

5

B.   [2S-(2R*,5R*,8R*,11R*)]-2,5,8,11,-Tetramethyl-
        1,4,7,10-tetraazacylcododecane-1,4,7-triacetic
   acid, gadolinium salt

10   A sample of Compound A (0.125 g) was dissolved
in 9 mL deionized water (reactant I). In a separate
vial, 0.129 g of GdCl$_3$ powder was dissolved in 1.5 mL
deionized water (reactant II). Reactants I and II
were treated as described in the method of Example 7F
15 to yield 80% of the title compound.
Mass Spectrum (FAB, m/e): (Gd^{159} + H)$^+$ at 559.
Elemental Analysis (C,H,N):
Calculated for C$_{18}$H$_{31}$N$_4$O$_6$Gd·2.81 H$_2$O: C 35.60, H
6.08, N 9.22%.
20 Found: C 35.65, H 6.11, N 9.02%.

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Example 10

10-(Phosphonomethyl)-1,4,7,10-tetraaza-
cyclododecane-1.4.7-triacetic acid, gadolinium salt

5
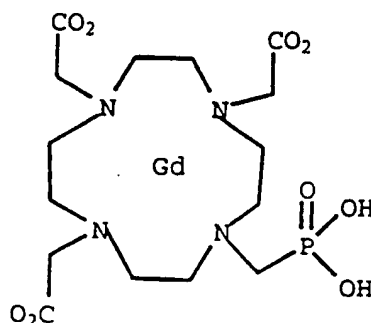
A.   10-(Phosphonomethyl)-1,4,7,10-tetraazacyclo-
dodecane-1.4.7-triacetic acid

10   A mixture of DO3A (1g, 2.89 mmol), phosphorus
acid (0.485 g, 5.91 mmol), concentrated hydrochloric
acid (1.1 mL, 13.4 mmol) and formaldehyde (1.35 mL of
37% aqueous solution, 16.4 mmol) in water (2.5 mL)
was refluxed for 27.5 hours. Removal of water from
15   the reaction mixture *in vacuo* gave an off-white solid
(1.74 g) as a crude product of Compound A. The solid
was dissolved in water (50 mL), and the pH of the
resulting solution was adjusted to 5.0 by addition of
1.0 N sodium hydroxide. The aqueous solution was
20   applied to a strong cation exchange column of AG50WX8
(250 mL, flowrate, 12.5 mL/minute). Initially, water
was used to remove any negatively charged inorganic
species, and ammonium hydroxide (1.0 M) was employed
to bring out the crude product. After removal of the
25   ammonium hydroxide from the fractions containing the
product, the residue dissolved in 5mM TEAB (pH 7.5)
was applied to a DEAE Sephadex ion exchange column
(750 mL, flowrate, 4 mL/minute). The column was

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eluted with TEAB, whose concentration varied from 5
to 400 mM. The concentration of the buffer was
doubled at every column volume (1L): 5, 10, 20, 40,
67, 80, 100, 125, 200 and 400 mM. Compound A
5   required 400 mM TEAB to be brought out from the
column. Evaporation of the buffer from the fractions
containing Compound A gave a white residue (0.9 g).
The solid, dissolved in water (35 mL) was loaded on a
strongly basic anion column of Amberlite IRA 900 C
10   (200 mL, flowrate, 14 mL/minute). Water was first
used to remove triethylamine followed by sulfuric
acid (1M) to elute Compound A from the column. The
fractions containing the product were combined and
applied to a column of Reillex 425
15   poly(4-vinyl)pyridine (PVP) 250 mL, 10 mL/minute).
Water was used to elute the product free from
sulfuric acid. Removal of water from the eluate (3.7
L) yielded Compound A (0.6 g, 47%) as a dense solid.

20   B.   10-(Phosphonomethyl)-1,4,7,10-tetraaza
             cyclododecane-1,4,7-triacetic acid, gadolinium
             salt

        A sample of Compound A (0.2 g) was dissolved
in 5 mL deionized water and its pH was adjusted to 4
25   with dilute NaOH. 0.096 g of Gd_2O_3 powder was added
slowly. The solution was refluxed overnight. The
excess metal ions were precipitated in the form of
$M(OH)_3 \cdot x H_2O$ by raising solution pH to about 9.5
with 1N NaOH and incubating at both about 70°C and
30   room temperature for several hours. The precipitate
was subsequently removed through centrifugation and
filtration with 0.22 μm membrane. The filtrate was
finally condensed and neutralized to pH 7 with 1N HCl
in preparation for HPLC purification. HPLC

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purification was performed as in Example 7F to yield
64% of the title compound.
Mass Spectrum (FAB, m/e): $(Gd^{159} + 2Na-H)^+$ at 640.
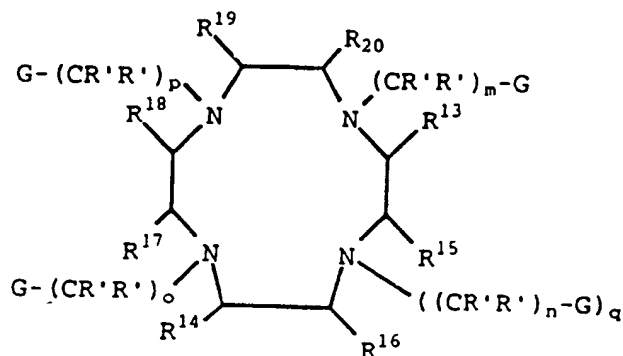Elemental Analysis (C,H,N): Calculated for
$C_{15}H_{24}N_4O_9PGdNa_2 \cdot 2.59\ H_2O$: C 26.29, H 4.29, N 8.18%.
Found: C 26.23, H 4.18, N 7.93%.

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What is claimed is:

1. A compound of the following formula I:

wherein

each m, n, o and p is independently 1 or 2;

q is 0 or 1;

each G is independently -COOR'', -P(O)(OR'')$_2$, -P(O)(OR'')(R'') or -C(O)N(R'')$_2$;

each R' is independently hydrogen or alkyl, alkoxy, cycloalkyl, hydroxyalkyl or aryl, each of which is optionally substituted, or a functional group capable of forming a conjugate with a biomolecule or of forming a multimer of said compound of formula I;

each R'' is hydrogen;

each R^{13} through R^{20} is independently hydrogen, alkyl, hydroxyalkyl, alkoxyalkyl or a functional group capable of forming a conjugate with a biomolecule or of forming a multimer of said compound of formula I;

or R^{13} together with R^{15}, and R^{17} together with R^{18}, independently form, together with the carbon atoms in the tetraazacyclododecane macrocycle to which they are attached, a fused fully or partially

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saturated non-aromatic cyclohexyl ring which may be
unsubstituted or substituted by one or more halogen,
alkyl, ether, hydroxy or hydroxyalkyl groups, and
which may be further fused to a carbocyclic ring, or

5   R^{13} and R^{15}, are each hydrogen and R^{17}, together with
R^{18}, forms a fused fully or partially saturated non-
aromatic cyclohexyl ring as defined above, or R^{13},
together with R^{15}, forms a fused fully or partially
saturated non-aromatic cyclohexyl ring as defined

10   above and R^{17} and R^{18} are hydrogen; provided that
(a.) when G is always -COOR'' and (i.) R', R'', R^{14}
and R^{16} through R^{20} are all hydrogen, then R^{13} and R^{15}
are other than hydrogen; (ii.) R'' and R^{13} through R^{20}
are all hydrogen, and m, n, o, p and q are each 1,

15   then (CR'R') is other than (CH_2) and ($CHCH_3$); (iii.)
R', R'', R^{13}, R^{14}, R^{17} and R^{20} are all hydrogen, then
at least two of R^{15}, R^{16}, R^{18} and R^{19} are other than
methyl; and (iv.) R'', R^{16}, R^{19} and R^{20} are all
hydrogen, and each (CR'R') is independently (CHR') or

20   (CH_2CHR'), then R^{13} and R^{15}, and R^{17} and R^{18}, are
other than a fused ring; and (b.) when G is always
-P(O)(OR'')$_2$, -P(O)(OR'')(R'') or -C(O)N(R'')$_2$, then
at least one R' or R^{13} through R^{20} is other than
hydrogen;

25   or a salt or multimeric form thereof.

2.   A metal chelate, comprising a compound of
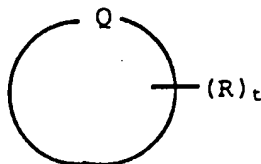claim 1 complexed with a metal atom.

30   3.   The chelate of claim 2, wherein the metal
is selected from atoms having an atomic number of 21
to 29, 42 or 57 to 83.

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4.. The chelate of claim 3, wherein said metal
is gadolinium.

5. A compound of the following formula II:

wherein
    Q is a 4- to an 8-membered carbocyclic ring
which may be fully or partially saturated;
    t is an integer from 2 to 16;
    each R group is independently hydrogen, -OH,
-CH$_2$-A, -OCH$_2$CH(OH)CH$_2$-A or a functional group
capable of forming a conjugate with a biomolecule,
provided that at least two of the R groups are
selected from -CH$_2$-A or -OCH$_2$CH(OH)CH$_2$-A; and
    A is a moiety capable of chelating a metal
atom.

6. A compound of Claim 5 wherein A is
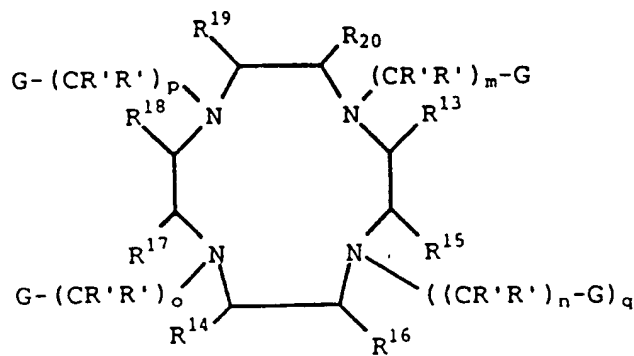
each m, n, o and p is independently 1 or 2;

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q is 0 or 1;

each G is independently -COOR'', -P(O)(OR'')$_2$,
-P(O)(OR'')(R'') or-C(O)N(R'')$_2$;

each R' is independently hydrogen or alkyl,
5   alkoxy, cycloalkyl, hydroxyalkyl or aryl, each of
which is optionally substituted, or a functional
group capable of forming a conjugate with a
biomolecule or of forming a multimer;

each R'' is hydrogen;

10   each R^{13} through R^{20} is independently
hydrogen, alkyl, hydroxyalkyl, alkoxyalkyl or a
functional group capable of forming a conjugate with
a biomolecule or of forming a multimer of said
compound of the formula I;

15   or R^{13} together with R^{15}, and R^{17} together with
R^{18}, independently form, together with the carbon
atoms in the tetraazacyclododecane macrocycle to
which they are attached, a fused fully or partially
saturated non-aromatic cyclohexyl ring which may be
20   unsubstituted or substituted by one or more halogen,
alkyl, ether, hydroxy or hydroxyalkyl groups, and
which may be further fused to a carbocyclic ring, or
R^{13} and R^{15} are each hydrogen and R^{17}, together with
R^{18}, forms a fused fully or partially saturated non-
25   aromatic cyclohexyl ring as defined above, or R^{13},
together with R^{15}, forms a fused fully or partially
saturated non-aromatic cyclohexyl ring as defined
above and R^{17} and R^{18} are hydrogen; provided that
(a.) when G is always -COOR'' and (i.) R', R'', R^{14}
30   and R^{16} through R^{20} are all hydrogen, then R^{13} and R^{15}
are other than hydrogen; (ii.) R'' and R^{13} through R^{20}
are all hydrogen, and m, n, o, p and q are each 1,
then (CR'R') is other than (CH$_2$) and (CHCH$_3$); (iii.)
R', R'', R^{13}, R^{14}, R^{17} and R^{20} are all hydrogen, then

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at least two of R^{15}, R^{16}, R^{18} and R^{19} are other than
methyl; and (iv.) R'', R^{16}, R^{19} and R^{20} are all
hydrogen, and each (CR'R') is independently (CHR') or
(CH$_2$CHR'), then R^{13} and R^{15}, and R^{17} and R^{18}, are

5   other than a fused ring; and (b.) when G is always
-P(O)(OR'')$_2$, -P(O)(OR'')(R'') or -C(O)N(R'')$_2$, then
at least one R' or R^{13} through R^{20} is other than
hydrogen;

       or a salt or multimeric form thereof.

10

       7. A compound of claim 5, wherein A is
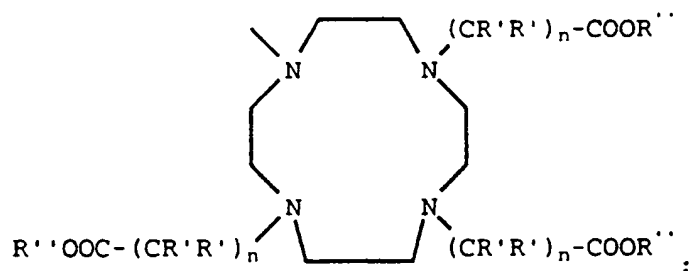
15   each R' is independently hydrogen, alkyl,
alkoxy, hydroxyalkyl, aryl, aralkyl or arylalkoxy;
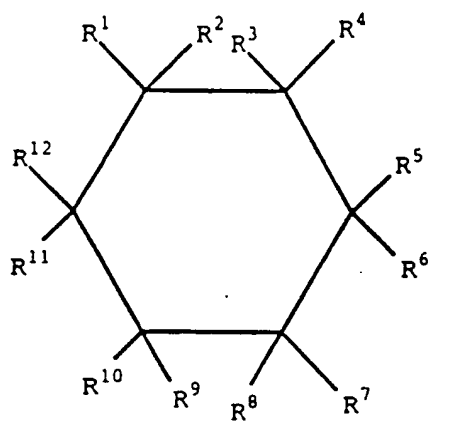   each R'' is hydrogen; and
       each n is 1 or 2.

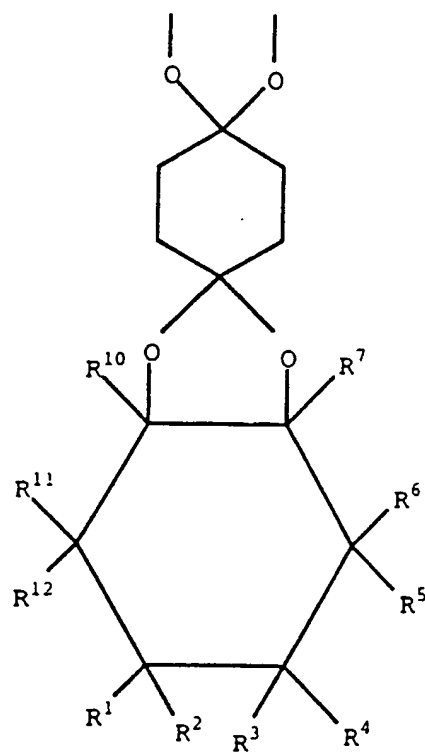20   8. A compound of claim 5 wherein

       is

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each R^1 through R^{12} group is independently
hydrogen, -OH, -CH$_2$-A, -OCH$_2$CH(OH)CH$_2$-A or a
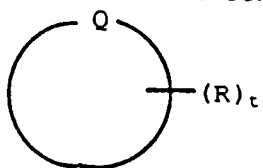functional group capable of forming a conjugate with
a biomolecule;

at least two of R^1 through R^{12} are selected
from -CH$_2$-A or -OCH$_2$CH(OH)CH$_2$-A; and

R^8 and R^9 taken together may additionally form
the group -O-[C(RR)]-O- where each R is independently
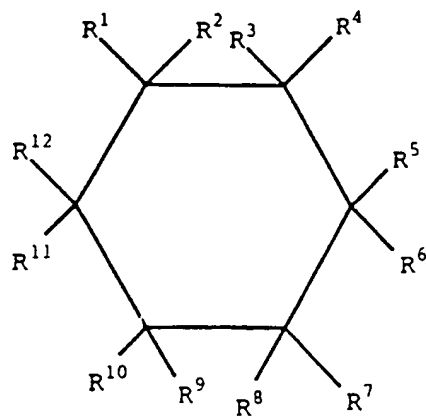hydrogen or alkyl, or R^8 and R^9 taken together may
form

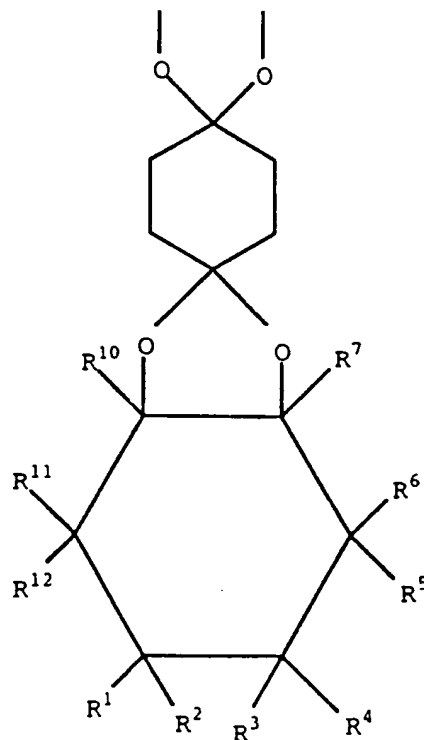9. A compound of claim 6 wherein
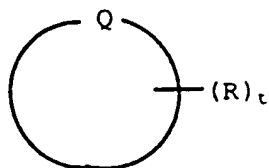

is a compound of the formula

5

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wherein

each R^1 through R^{12} group is independently hydrogen, -OH, $-CH_2-A$, $-OCH_2CH(OH)CH_2-A$ or a functional group capable of forming a conjugate with
5   a biomolecule;

at least two of R^1 through R^{12} are selected from $-CH_2-A$ or $-OCH_2CH(OH)CH_2-A$; and

R^8 and R^9 taken together may additionally form the group $-O-[C(RR)]-O-$ where each R is independently
10   hydrogen or alkyl, or R^8 and R^9 taken together may form

10.   A compound of claim 7, wherein

15   is

each R^1 through R^{12} group is independently
hydrogen, $-OH$, $-CH_2-A$, $-OCH_2CH(OH)CH_2-A$ or a
functional group capable of forming a conjugate with
a biomolecule;

at least two of R^1 through R^{12} are selected
from $-CH_2-A$ or $-OCH_2CH(OH)CH_2-A$; and

R^8 and R^9 taken together may additionally form
the group $-O-[C(RR)]-O-$ where each R is independently
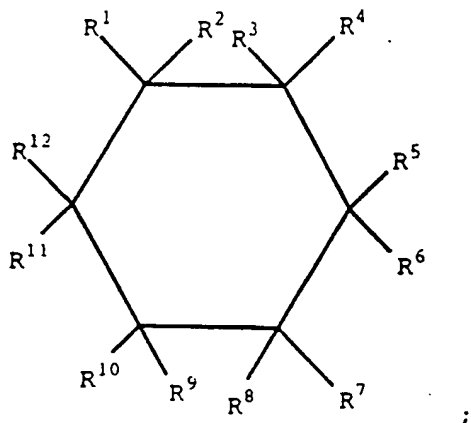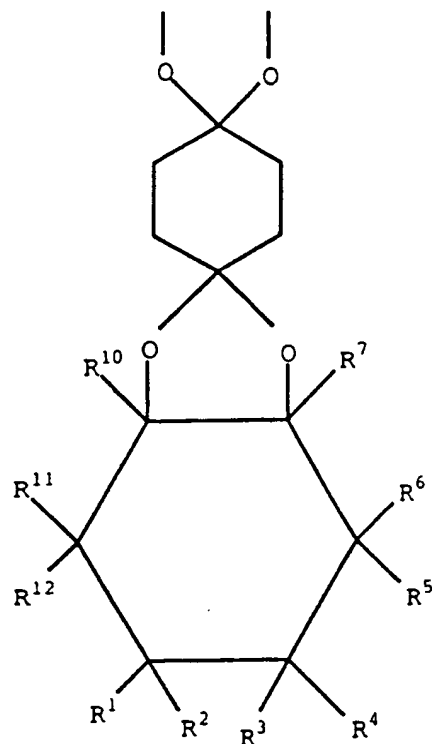hydrogen or alkyl, or R^8 and R^9 taken together may
form

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11. A metal chelate, comprising a compound of claim 5 complexed with a metal atom.

12. A metal chelate, comprising a compound of claim 6 complexed with a metal atom.

13. A conjugate, comprising a compound of claim 5 conjugated with a biomolecule.

14. A metal chelate, comprising a conjugate of claim 13 complexed with a metal atom.

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15.   A method for diagnostic imaging,
comprising the steps of administering to a host a
compound of claim 5, which compound is complexed with
a metal, and obtaining a diagnostic image of said
5   host.

16.   The method of claim 15, wherein said
image is a magnetic resonance image.

10   17.   A method for diagnostic imaging,
comprising the steps of administering to a host a
conjugate of claim 13, which conjugate is complexed
with a metal, and obtaining a diagnostic image of
said host.
15

18.   A compound selected from the group
consisting of:

(1α,2α,4ß,5ß)-10,10'-[(1,2,4,5-Tetrahydroxy-
20   1,4-cyclohexanediyl)bis(methylene)]bis[1,4,7,10-
tetraazacyclododecane-1,4,7-triacetic acid];

(3aα,4α,5β,6α,7β,7aα)-10,10'10",10"'-[[Hexahydro-2,2-
dimethyl-1,3-benzodioxol-4,5,6,7-tetrayl]tetra(oxy)-
25   tetra(2-hydroxy-3,1-propanediyl)]tetrakis[1,4,7,10-
tetraazacyclododecane-1,4,7-triacetic acid];

3,4,5,6-Tetra-O-[2-hydroxy-3-[4,7,10-
tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-
30   yl]propyl]-myo-inositol;

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(1α,2α,3α,4β,5α,6β)-10,10'-[(2,3,5,6-tetrahydroxy-
1,4-cyclohexanediyl)bis(2-hydroxy-3,1-propanediyl)]-
bis[1,4,7-10-tetraazacyclododecane-1,4,7-triacetic
acid];

10-[[1,4-Dihydroxy-2,5-bis[2-hydroxy-3-[4,7,10-tris-
(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-
propoxy]-4-[[4,7,10-tris(carboxymethyl)-1,4,7,10-
tetraazacyclododecan-1-yl]methyl]cyclohexyl]methyl]-
1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid;

[3aR-(3aα,3'aα,4ß,4'ß,5α,5'α,6ß,6'b,7α,-
7'α,7aα,7'aα)]-dodecahydro-4,4',5,5',6,6',7,7'-
octakis-[[2-hydroxy-3-[(4,7,10-tricarboxymethyl)-
1,4,7,10-tetraazacyclododecan-1-yl]propyl]-
oxy]dispiro-[1,3-benzodioxole-2,1'-cyclo-hexane-
4',2'-[1,3]-benzodioxole];

[2S-(2α,5α,8α,11α,)]-2,5,8,11-Tetramethyl-1,4,7,10-
tetraazacyclododecane-1,4,7,10-tetraacetic acid;

[αR—(aR*,α'R*,α'R*,α'''R*,2S*,5S*,8S*,11S*)]-α,-
α',α',a''',2,5,8,11-Octamethyl-1,4,7,10-
tetraazacyclododecane-1,4,7,10-tetraacetic acid;

[2S-(2R*,5R*,8R*,11R*)]-2,5,8,11,-Tetramethyl-
1,4,7,10-tetraazacylcododecane-1,4,7,-triacetic acid;
and

10-(Phosphonomethyl)-1,4,7,10-tetraaza-
cyclododecane-1,4,7-triacetic acid.

19. A compound selected from the group
consisting of:

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(1α,2α,4ß,5ß)-10,10'-[(1,2,4,5-Tetrahydroxy-
1,4-cyclohexanediyl)bis(methylene)]bis[1,4,7,10-
tetraazacyclododecane-1,4,7-triacetic acid],
digadolinium salt;

5

(3aα,4α,5β,6α,7β,7aα)-10,10'10',10''-[[Hexahydro-2,2-
dimethyl-1,3-benzodioxol-4,5,6,7-tetrayl]tetra(oxy)-
tetra(2-hydroxy-3,1-propanediyl)]tetrakis[1,4,7,10-
tetraazacyclododecane-1,4,7-triacetic acid],
10   tetragadolinium salt;

3,4,5,6-Tetra-O-[2-hydroxy-3-[4,7,10-
tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-
yl]propyl]-myo-inositol, tetragadolinium salt;

15

(1α,2α,3α,4β,5α,6β)-10,10'-[(2,3,5,6-tetrahydroxy-
1,4-cyclohexanediyl)bis(2-hydroxy-3,1-propanediyl)]-
bis[1,4,7-10-tetraazacyclododecane-1,4,7-triacetic
acid], digadolinium salt;

20

10-[[1,4-Dihydroxy-2,5-bis[2-hydroxy-3-[4,7,10-tris-
(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]-
propoxy]-4-[[4,7,10-tris(carboxymethyl)-1,4,7,10-
tetraazacyclododecan-1-yl]methyl]cyclohexyl]methyl]-
25   1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid,
tetragadolinium salt;

[3aR-(3aα,3'aα,4ß,4'ß,5α,5'α,6ß,6'b,7α,-
7'α,7aα,7'aα)]-dodecahydro-4,4',5,5',6,6',7,7'-
30   octakis-[[2-hydroxy-3-[(4,7,10-tricarboxymethyl)-
1,4,7,10-tetraazacyclododecan-1-yl]propyl]-
oxy]dispiro-[1,3-benzodioxole-2,1'-cyclo-hexane-
4',2'-[1,3]-benzodioxole], octagadolinium salt;

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[2S-(2α,5α,8α,11α,)]-2,5,8,11-Tetramethyl-1,4,7,10-
tetraazacyclododecane-1,4,7,10-tetraacetic acid,
gadolinium salt;

5   [αR-(aR*,α'R*,α*R*,α'''R*,2S*,5S*,8S*,11S*)]-α,-
α',α*,a''',2,5,8,11-Octamethyl-1,4,7,10-
tetraazacyclododecane-1,4,7,10-tetraacetic acid,
gadolinium salt;

10   [2S-(2R*,5R*,8R*,11R*)]-2,5,8,11,-Tetramethyl-
1,4,7,10-tetraazacylcododecane-1,4,7-triacetic acid,
gadolinium salt; and

10-(Phosphonomethyl)-1,4,7,10-tetraaza-
15   cyclododecane-1,4,7-triacetic acid, gadolinium salt.

20.   A chelate other than [1R-
(1R*,4R*,7R*,10R*)]-α,α',α'',α'''-tetramethyl-
1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic
20   acid or [1R-(1R*,4R⁻,7R*)]-α,α',α''-trimethyl-
1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid
possessing a stability greater than 10^{15} M^{-1} and
capable of exhibiting an immobilized relaxivity
between about 60 and 200 $mM^{-1}s^{-1}$/metal atom.
25

21.   A chelate of claim 20 capable of
exhibiting an immobilized relaxivity between about 70
and 150 $mM^{-1}s^{-1}$/metal atom.

30   22.   A chelate of claim 20 capable of
exhibiting an immobilized relaxivity between about 80
and 100 $mM^{-1}s^{-1}$/metal atom.

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23.   A chelate possessing a stability greater than $10^{15}\, M^{-1}$ and capable of exhibiting an immobilized relaxivity between about 60 and 200 $mM^{-1}s^{-1}$/metal atom comprising a compound of claim 1.

5

24.   A chelate possessing a stability greater than $10^{15}\, M^{-1}$ and capable of exhibiting an immobilized relaxivity between about 70 and 150 $mM^{-1}s^{-1}$/metal atom comprising a compound of claim 1.

10

25.   A chelate possessing a stability greater than $10^{15}\, M^{-1}$ and capable of exhibiting an immobilized relaxivity between about 80 and 100 $mM^{-1}s^{-1}$/metal atom comprising a compound of claim 1.

15

26.   A chelate possessing a stability greater than $10^{15}\, M^{-1}$ and capable of exhibiting an immobilized relaxivity between about 60 and 200 $mM^{-1}s^{-1}$/metal atom comprising a compound of claim 8.

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27.   A chelate possessing a stability greater than $10^{15}\, M^{-1}$ and capable of exhibiting an immobilized relaxivity between about 70 and 150 $mM^{-1}s^{-1}$/metal atom comprising a compound of claim 8.

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28.   A chelate possessing a stability greater than $10^{15}\, M^{-1}$ and capable of exhibiting an immobilized relaxivity between about 80 and 100 $mM^{-1}s^{-1}$/metal atom comprising a compound of claim 8.

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29.   A chelate other than [1R-(1R*,4R*,7R*,10R*)]-$\alpha,\alpha',\alpha'',\alpha'''$-tetramethyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid or [1R-(1R*,4R*,7R*)]-α,α',α''-trimethyl-

1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, including an immobilized functional group, capable of exhibiting an immobilized relaxivity between about 60 and 200 $mM^{-1}s^{-1}$/metal atom.

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30. A chelate of claim 29, capable of exhibiting an immobilized relaxivity between about 70 and 150 $mM^{-1}s^{-1}$/metal atom.

10   31. A chelate of claim 29, capable of exhibiting an immobilized relaxivity between about 80 and 100 $mM^{-1}s^{-1}$/metal atom.

32. A chelate including a functional group,
15 capable of exhibiting an immobilized relaxivity between about 60 and 200 $mM^{-1}s^{-1}$/metal atom comprising a compound of claim 1.

33. A chelate including a functional group,
20 capable of exhibiting an immobilized relaxivity between about 70 and 150 $mM^{-1}s^{-1}$/metal atom comprising a compound of claim 1.

34. A chelate including a functional group,
25 capable of exhibiting an immobilized relaxivity between about 80 and 100 $mM^{-1}s^{-1}$/metal atom comprising a compound of claim 1.

35. A chelate including a functional group,
30 capable of exhibiting an immobilized relaxivity between about 60 and 200 $mM^{-1}s^{-1}$/metal atom comprising a compound of claim 8.

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36. A chelate including a functional group,
capable of exhibiting an immobilized relaxivity
between about 70 and 150 $mM^{-1}s^{-1}$/metal atom
comprising a compound of claim 8.

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37. A chelate including a functional group,
capable of exhibiting an immobilized relaxivity
between about 80 and 100 $mM^{-1}s^{-1}$/metal atom
comprising a compound of claim 8.

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38. A chelate other than [1R-
(1R*,4R*,7R*,10R*)]-α,α',α'',α'''-tetramethyl-
1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic
acid, conjugated to a biomolecule, capable of
15   exhibiting an immobilized relaxivity between about 60
and 200 $mM^{-1}s^{-1}$/metal atom.

39. A chelate of claim 38, capable of
exhibiting an immobilized relaxivity between about 70
20   and 150 $mM^{-1}s^{-1}$/metal atom.

40. A chelate of claim 38, capable of
exhibiting an immobilized relaxivity between about 80
and 100 $mM^{-1}s^{-1}$/metal atom.

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41. A chelate conjugated to a biomolecule,
capable of exhibiting an immobilized relaxivity
between about 60 and 200 $mM^{-1}s^{-1}$/metal atom
comprising a compound of claim 1.

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42. A chelate conjugated to a biomolecule,
capable of exhibiting an immobilized relaxivity
between about 70 and 150 $mM^{-1}s^{-1}$/metal atom
comprising a compound of claim 1.

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43. A chelate conjugated to a biomolecule,
capable of exhibiting an immobilized relaxivity
between about 80 and 100 $mM^{-1}s^{-1}$/metal atom
comprising a compound of claim 1.

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44. A chelate conjugated to a biomolecule,
capable of exhibiting an immobilized relaxivity
between about 60 and 200 $mM^{-1}s^{-1}$/metal atom
comprising a compound of claim 8.

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45. A chelate conjugated to a biomolecule,
capable of exhibiting an immobilized relaxivity
between about 70 and 150 $mM^{-1}s^{-1}$/metal atom
comprising a compound of claim 8.

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46. A chelate conjugated to a biomolecule,
capable of exhibiting an immobilized relaxivity
between about 80 and 100 $mM^{-1}s^{-1}$/metal atom
comprising a compound of claim 8.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6   C07D257/02   C07D493/10   C07D405/14   A61K49/00   C07F9/6524
//(C07D493/10,317:00,317:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6   C07D   A61K   C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 382 583 (CELLTECH LIMITED) 16 August 1990 *Complete document* ---	1-4,6,7, 15-46
A	EP,A,0 365 412 (GUERBET S.A.) 25 April 1990 *Complete document* ---	1-4,6,7, 15-46
A	EP,A,0 485 045 (SCHERING AKTIENGESELLSCHAFT) 13 May 1992 *Complete document* *Page 28-29: example 21 ,sub b* -----	5,7-46

☐ Further documents are listed in the continuation of box C.   ☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 July 1995	27. 07. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Luyten, H

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